The American Journal of Medicine

November 1955

The American Journal of Medicine

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1. Winsor, T., and Humphreys, P.: Angiology *3*:1 (Feb.) 1952. 2. Plotz, M.: New York State J. Med. *52*:2012 (Aug. 15) 1952. 3. Dailheu-Geoffroy, P.: L'Ouest-Médical, vol. 3 (July) 1950.

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Clinical Studies

Acute Fatty Metamorphosis of the Liver Associated with Pregnancy. A Distinctive Lesion . . . . . WILLIAM B. OBER AND PHILIP M. LECOMPTE 743

In a study of unusual interest the authors describe cases of what appears to be a distinct form of acute fatty metamorphosis of the liver, not to be confused with acute yellow liver atrophy, occasionally occurring in the last trimester of pregnancy. The increase in lipid content of the liver, also of the renal tubular epithelium, consists of fatty acids. The authors speculate upon the pathogenesis of this disorder and suggest that, in view of the similarities with ethionine poisoning, some aberration of metabolism, possibly of transmethylation, is responsible.

Disturbances of Impulse Formation and Conduction in the Pre-excitation (WPW) Syndrome—Their Bearing on Its Mechanism . ALFRED PICK AND LOUIS N. KATZ 759

The controversy over the nature of the ventricular pre-excitation characteristic of the Wolff-Parkinson-White syndrome continues unabatedly, particularly as to whether it should be attributed to a hyperexcitable ventricular focus or to conduction along accessory, by-passing pathways. The present interesting study attacks the problem by detailed analysis of associated arrhythmias. The authors demonstrate that by making a sufficient number of plausible assumptions and by exercising extraordinary ingenuity it is possible to account for all of a great variety of electrocardiographic

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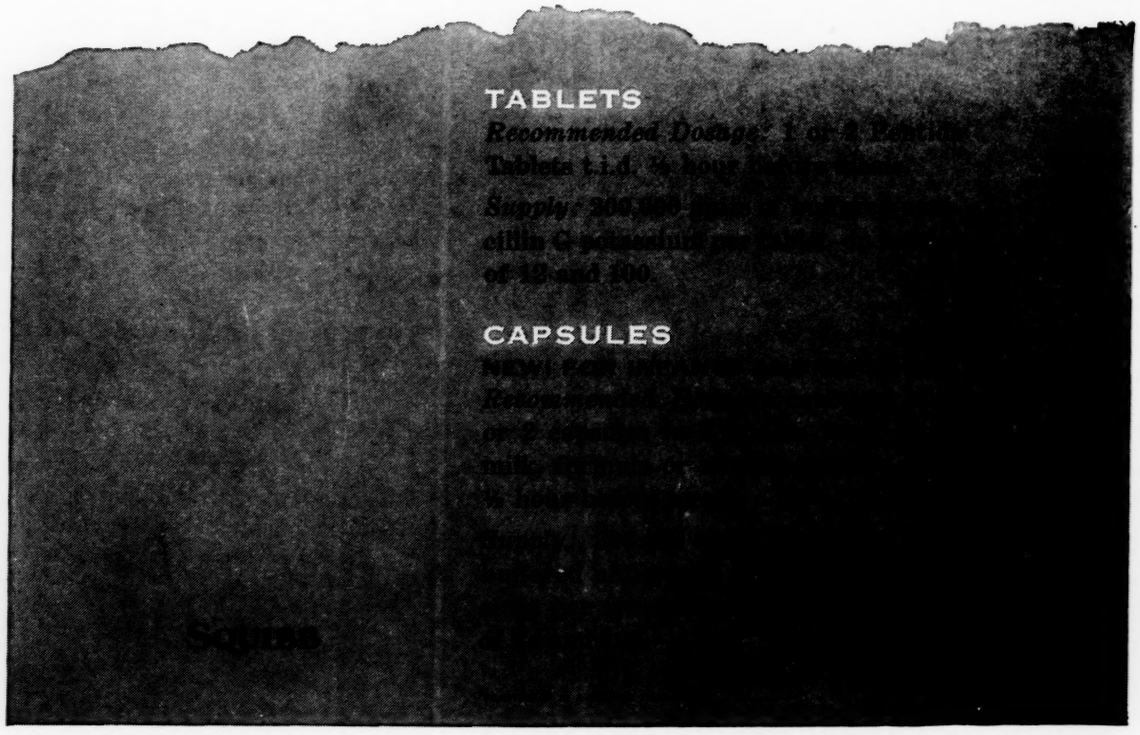

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anomalies on the basis of anomalous A-V pathways, without resorting to the presumption of any hyperexcitable ventricular focus. As an intellectual *tour de force*, this is certainly an impressive contribution to the subject.

Gross Hematuria in Sickle Cell Trait and Sickle Cell Hemoglobin-C Disease
A. Zerne Chapman, Paul S. Reeder, Irving A. Friedman and Lyle A. Baker 773

The role of sickling as a cause of gross hematuria, without other evidences of systemic disease, has been somewhat neglected. Bleeding from the kidney may occur with the heterozygous trait, as this study indicates. The possibilities in relation to hemoglobin-C disease, and other hemoglobin anomalies, are unusually interesting, as brought out here.

Adrenal Hormone Therapy in Viral Hepatitis. IV. The Effect of Gamma Globulin and Oral Cortisone in the Acute Disease
Alfred S. Evans, Col. Robert S. Nelson, Lt. Col. Helmuth Sprinz and Capt. Frank P. Cantrell 783

The place of ACTH and cortisone in the treatment of viral hepatitis is still uncertain. In this study cortisone by mouth caused a rapid initial drop in bilirubinemia but did not shorten the total duration of illness in most cases. Gamma globulin was given concurrently, because previous experience indicated a higher relapse rate when hepatitis was treated with ACTH or cortisone (due presumably to interrupted development of antibody response) but without decrease in the relapse rate. The authors concluded that cortisone is not indicated in routine management of acute viral hepatitis unless fulminant or refractory.

Seminar on Carbohydrate Metabolism

Experimental Diabetes and Its Relation to Diabetes Mellitus . . F. D. W. Lukens 790

Dr. Lukens contributes a stimulating discussion of the significance of the observations in experimental diabetes in relation to the naturally occurring phenomena of diabetes mellitus in man. This is a topic that has aroused some uneasiness and confusion among clinicians, consequently Dr. Lukens' authoritative views are especially welcome. The discussion leaves no question as to the contribution made by the experimental production of diabetes in animals to our understanding of the human disorder and to the more explicit further study of the disease in man. Of special interest are the reflections upon appraisal of the severity of the metabolic derangements in diabetes mellitus and on the cause(s) of the disease in human subjects.

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Case Reports

Treatment of Scleroderma, Sclerodactylia and Calcinosis by Chelation (EDTA)
RUBIN KLEIN AND SAMUEL B. HARRIS 798

The striking improvement noted in this case of scleroderma and calcinosis treated with EDTA intravenously would seem to exceed that which may occur spontaneously. Despite previous cruel disappointments in the therapy of this disabling disorder, the procedure described deserves further trial.

Phrenic Paralysis Due to Serum Neuritis . HUGH P. SMITH AND HUGH P. SMITH, JR. 808

The authors make a strong case for their thesis, that paralysis of the hemidiaphragm in otherwise healthy persons not harboring any of the recognized causes of phrenic paralysis should suggest serum neuritis, and particularly tetanus antitoxin serum neuritis, as the cause. Four probable cases are cited.

Herpetic Meningo-encephalitis Accompanying Cutaneous Herpes Simplex
BREWSTER P. HUNT AND EDWARD O'B. COMER 814

An interesting and well studied case of multiple cutaneous lesions due to herpes simplex, with complicating herpetic meningo-encephalitis. The diagnosis was made in the face of difficulties which might well have thrown most physicians off the track. The authors comment on some of the clinical and laboratory problems encountered in establishing this diagnosis.

Fibrinolytic Purpura in Acute Leukemia
ANTHONY V. PISCIOTTA AND EARL J. SCHULZ 824

The role of proteolytic enzymes which dissolve fibrin clots in patients with acute leukemia and so contribute to hemorrhage in this disorder deserves further exploration, as this interesting report indicates.

Advertising Index on 3rd Cover

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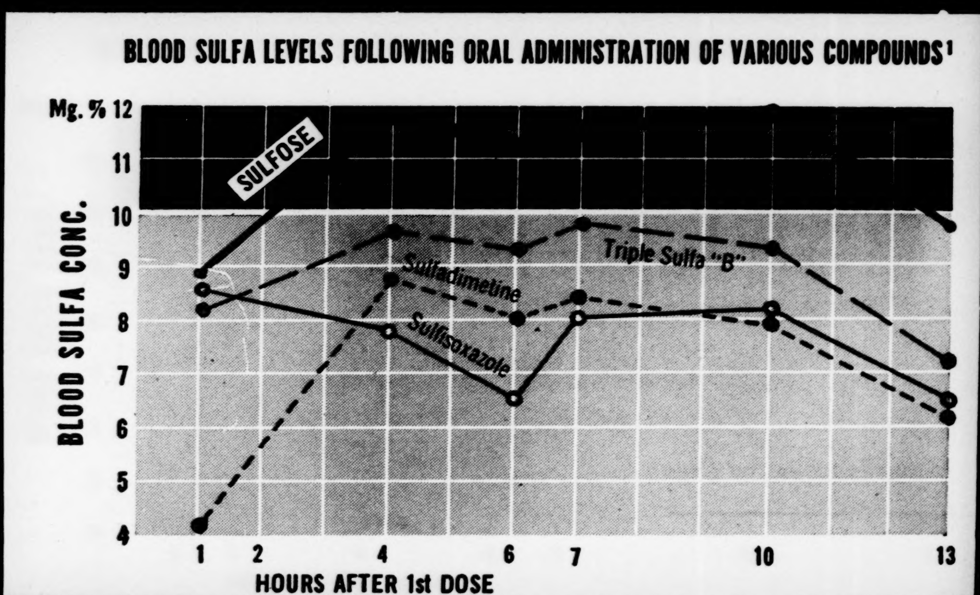

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1. Berkowitz, D.: Antibiot. & Chemo. (June)

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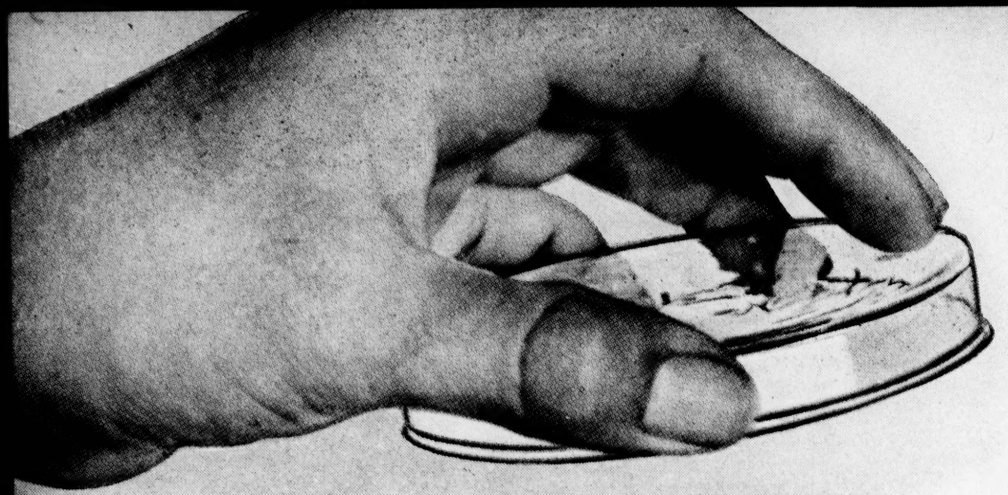

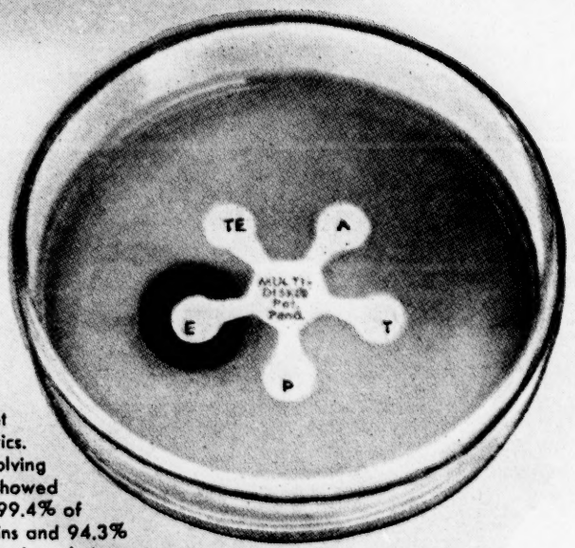

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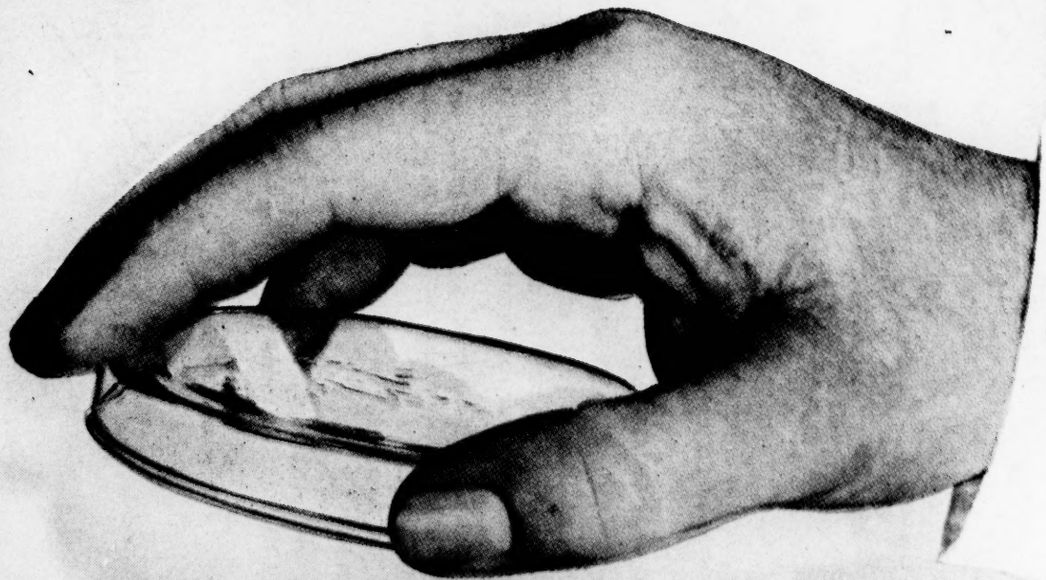

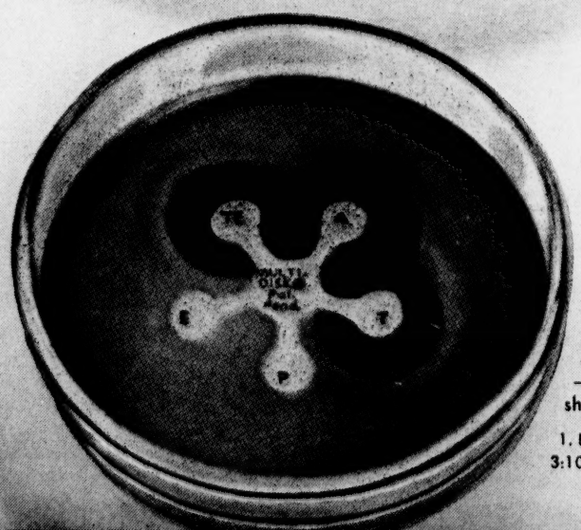

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1. Eisenberg, et al., Antib. & Chemo., 3:1026-1028, Oct., 1953.

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1. Eisfelder, H.W.: Am. Pract. & Dig. Treat., 5:778 (Oct.) 1954. 2. Sebrell, W.H., Jr.: J.A.M.A. 152:42 (May) 1953. 3. Sherman, R.J.: Medical Times, 82:107 (Feb.) 1954.

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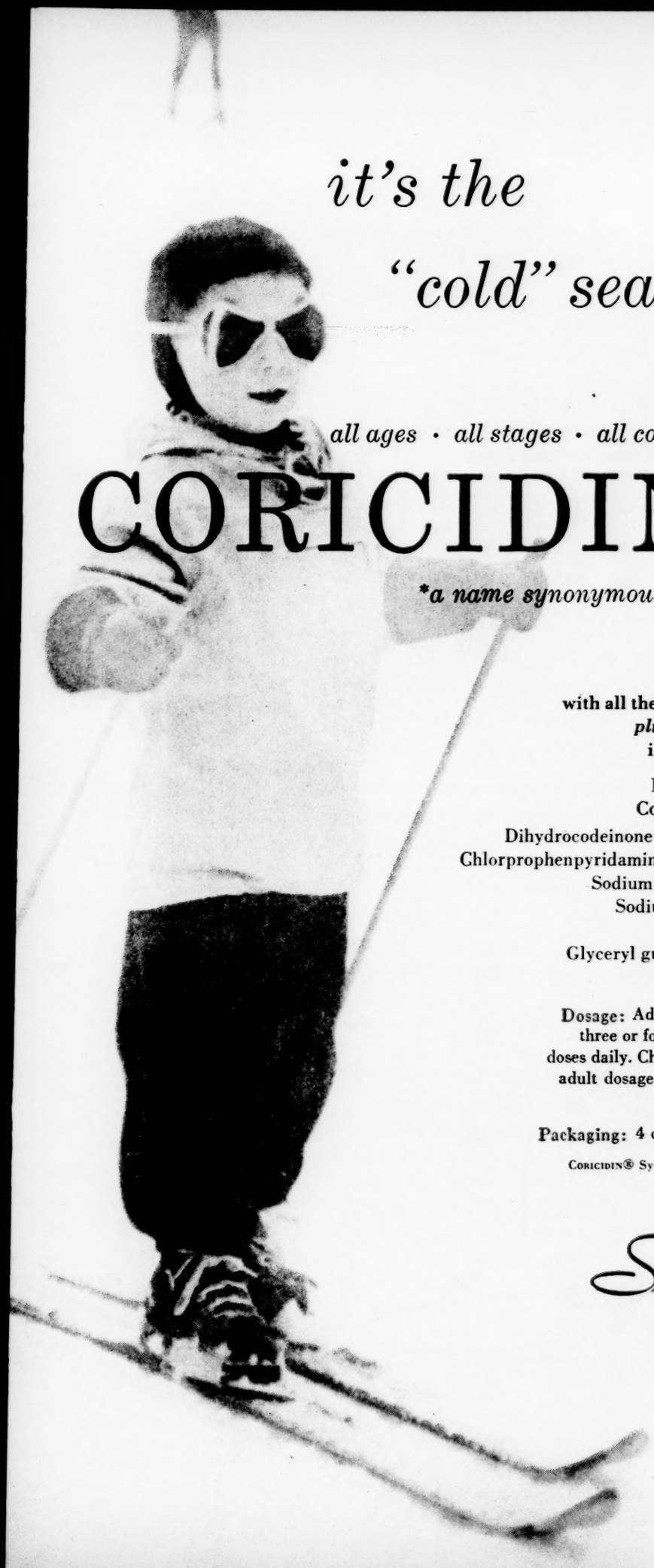

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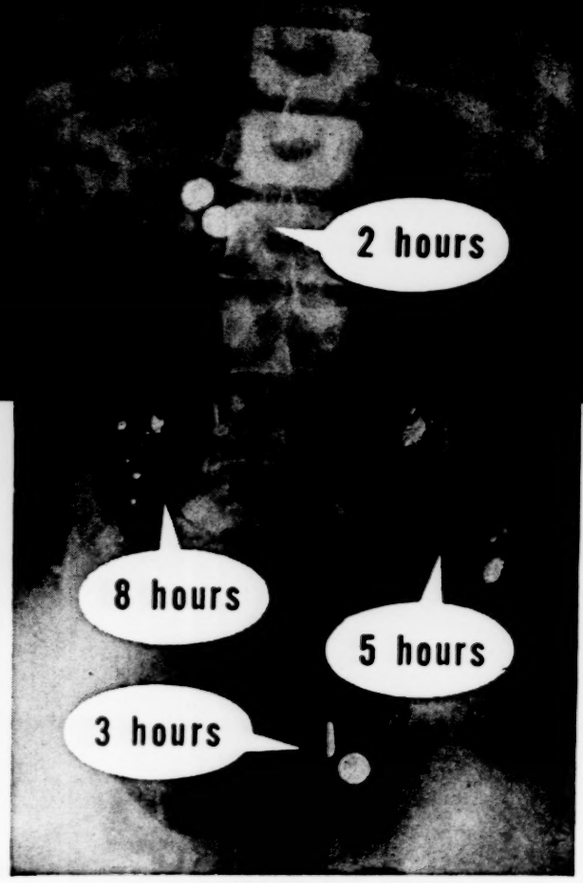

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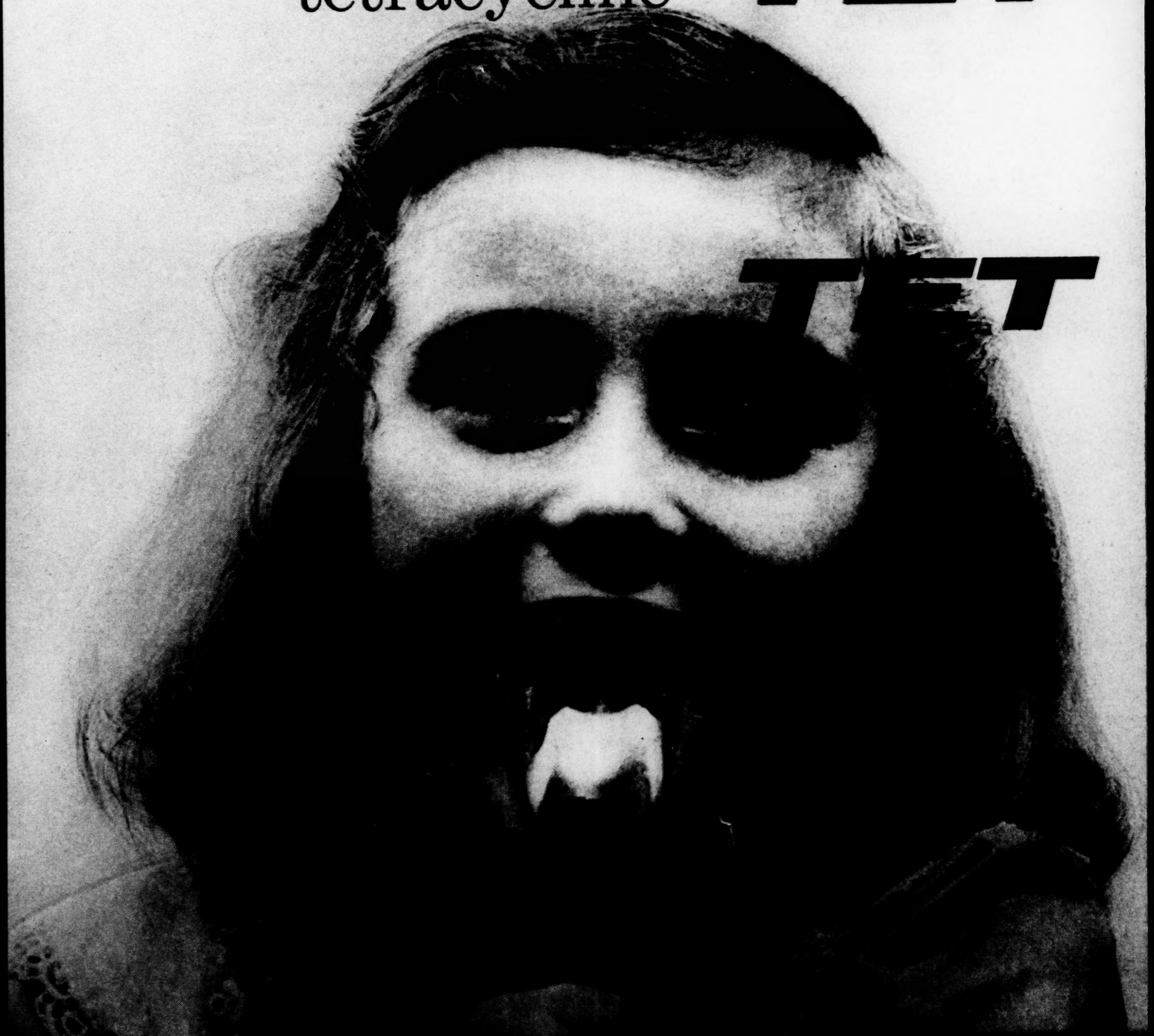

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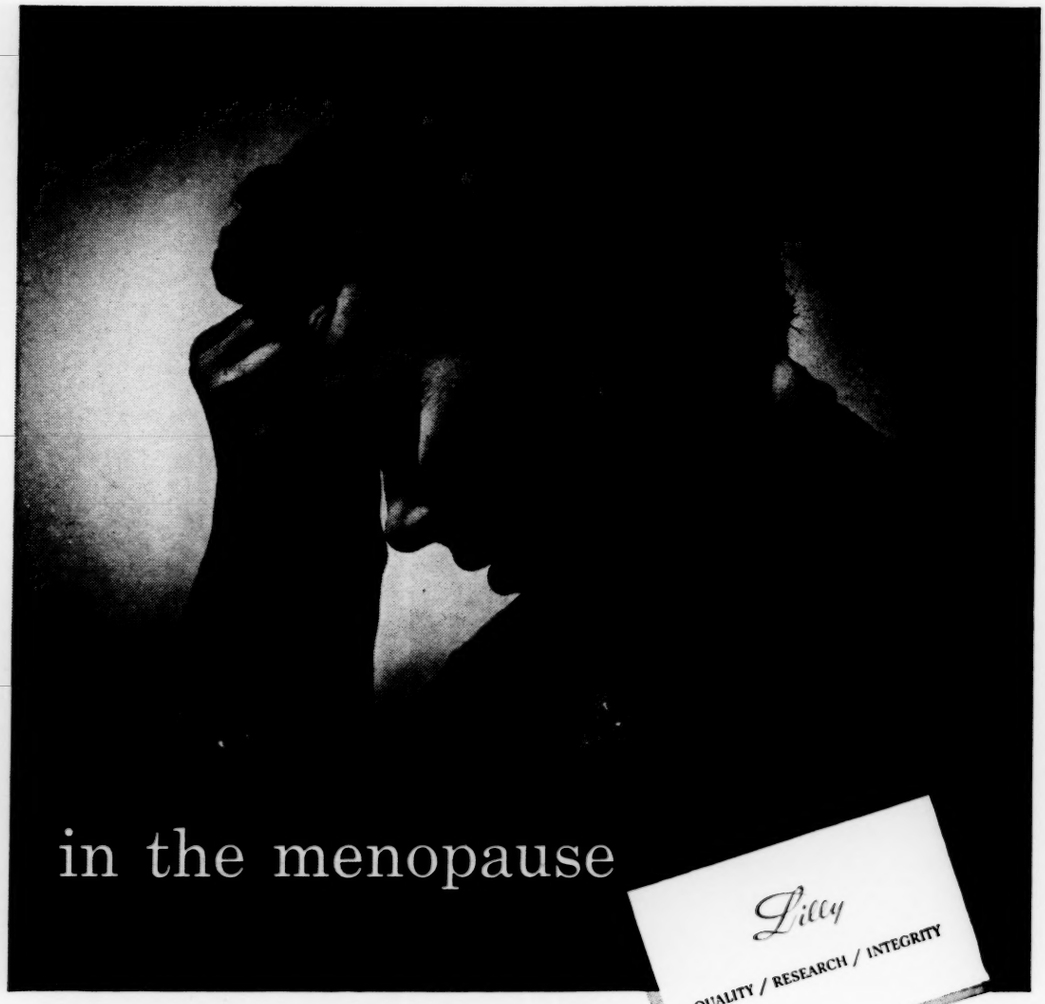

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The American Journal of Medicine

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Foreword

THIS conference on myasthenia gravis sponsored by the Myasthenia Gravis Foundation was held at the Medical School of the University of Pennsylvania on December 8 and 9, 1954. Presiding at the various sessions were Dr. Henry Viets, Chairman of the Medical Board of the Foundation, Dr. Elmer Severinghaus and Dr. George Morris Piersol, Dean of the Graduate School of the University of Pennsylvania.

The objectives of the conference were conceived as a review of current investigations on the disease and its therapy, to summarize present concepts of the physiology of neuromuscular activity and to discuss directions of future study. In several papers the influence of endocrine factors on neuromuscular function in normal states and in this disease was examined. Dr. Venning in her review on the endocrine changes in pregnancy considers whether or not such alterations might bear on remissions of the disease sometimes occurring in pregnancy.

The papers are divided into sections dealing with the various aspects of this subject. The first includes a statement of the clinical problem by Dr. Viets and a review of normal neuromuscular transmission; the others discuss the pharmacology of neuromuscular transmission, the pathologic physiology of myasthenia, the thymus and myasthenia, the endocrine glands and myasthenia, and the drug therapy and management of the disease.

This introduction attempts to call attention to some of the chief points brought out in the discussion. All except two of the original papers are published here. Space limitations unfortunately precluded the use of most of the discussion except in three instances where factual information of importance was added.

The Foundation wishes to acknowledge the cooperation and generosity of Mr. Elmer Bobst, Ciba Pharmaceutical Products, Hoffmann-La Roche, Inc., Smith, Kline and French Laboratories and Winthrop-Stearns, Inc.

DISCUSSION OF PAPERS

The modern era in the study of this disease began about 1935 with the neurohumoral theory of Loewi and Dale and the realization that the disease, which resembled curare poisoning, could be reversed by the antidote, eserine, and later by prostigmin. These observations led to three lines of study: (1) A comparison was made, by electromyographic means, of the disease with the normal process and with the blocking effect of curare and other agents. (2) Because of the analogy to curare poisoning, efforts were made to discover a circulating toxin, especially directed toward the thymus gland as a source. (3) A study of therapy with new drugs having anticurare or anticholinesterase activity was done.

Meantime, our knowledge of the process of normal nerve-muscle activity has been elaborated, and Drs. Nastuk, Koelle and Nachmansohn* reported some of the current developments in this field. The nature of the action of blocking agents was reviewed for us by Dr. Van Maanen and Dr. Churchill-Davidson and discussed by others. The myasthenic disability was compared to the normal process and to the action of these blocking agents by Drs. Johns, Grob and Harvey, Dr. Churchill-Davidson and Dr. Botelho.

Concerning the normal process, we have learned that acetylcholine is held in a bound form and released upon stimulation, whereby it effects a contraction of muscle. It is promptly broken down by the enzyme cholinesterase into choline and acetate and again resynthesized by another enzyme, choline-acetylase, into the bound form. The action of acetylcholine is to depolarize the membrane, which is accompanied

* Paper not included in this issue.

by shift of sodium into the cell and potassium out of the cell. Nastuk pointed out that this process is influenced by the composition of the extracellular fluid, being greatly impeded by withdrawal of sodium. He considers that acetylcholine does not act by shifting into the endplate because acetylcholine liberation is not influenced by a change in extracellular sodium, although the depolarization process is. Dr. Nachmansohn discussed the action of quaternary nitrogen compounds in terms of their binding properties by physiochemical action to the receptor proteins which he distinguishes from the specific enzyme proteins previously mentioned. In their relative binding he sees an explanation for the lesser effect of tertiary nitrogen compounds compared to the quaternary group. Dr. Koelle showed by histochemical means that the specific enzyme cholinesterase concentrates about the palisade-like subneural apparatus of the endplate. He also reported that this enzyme has now also been found in adrenergic nerves and posterior roots in small concentration, as had previously been known of the cholinergic nerves. He suggested that the endplate region should be studied by these methods and compared to the normal.

As a result of studies of blocking agents, two types have been described, namely, a depolarizing block and a non-depolarizing block. The studies of Drs. Johns, Grob and Harvey suggested that a continuing block exists in the resting myasthenic muscle, since its action potential is below normal. They consider this to act as a competitive type of block due to some substance liberated as part of the normal process, probably choline. They present evidence showing that the myasthenic abnormality can be duplicated by administration of choline. This seems to be an important advance in our concept of the nature of the defect in myasthenic muscle at its endplate. Presumably the view formerly held by these investigators that lessened sensitivity of the myasthenic endplate to acetylcholine constituted the major change in this disease has given way to this new concept.

Dr. Churchill-Davidson presented evidence showing that the action of decamethonium in the myasthenic differed from that in the normal subject. In the latter a depolarizing block occurs but in the former a combined depolarizing and non-depolarizing block develops. Unaffected muscle in myasthenics revealed resistance to depolarization. Dr. Grob raised the question as to whether or not a faster rate of stimulation than that employed might have given different results. I assume Dr. Churchill-Davidson accepts this point.

Two papers drew attention to the muscle itself in contrast to endplate blocking agents. Dr. Botelho reported that the tension curve is a better index of the weakness than the electromyograph which may not be affected in the same direction as the tension curve. Whether or not this is a defect of the myograph technic or a fault of the disease is open to question. Dr. Shy* reported his early studies of sodium and potassium concentration in intra- and extracellular water in human biopsy specimens. These have not yet shown any consistent pattern, but have not confirmed Cummings' findings of the accumulation of potassium in myasthenic muscle.

The theory that the thymus gland may contain and release a blocking or depressing substance in myasthenia gravis has been the object of study by Professor Andrew Wilson for several years. In summarizing his latest findings he noted that extracts of the thymus glands, especially from the fetal whale, contained a depressing substance. The action of these extracts does not resemble that of the ordinary blocking drugs and is not influenced by prostigmin. The nature of this material is unknown. Thymic extracts made by another technic, as pointed out by Dr. Zack, may contain potassium in amounts sufficient to account for some of the depression observed. The efforts of Dr. Rider to show a difference in the effect of thymic extracts from myasthenic and normal subjects, or of serum from myasthenic subjects failed to demonstrate any difference in cat neuromuscular preparations. Dr. Schwartz's animals on injection of thymus extract showed either convulsions or spasm along with vomiting, collapse and weakness. It would seem therefore that at the moment we are unable directly to relate the thymus, on the basis of this type of experimental work, to the production in animals of a condition resembling myasthenia gravis. Such a relationship still remains to be demonstrated. Dr. Van Maanan pointed out that the method of assay for blockading agents used in some of these studies may be too insensitive, for it does not show up curare in the serum of an animal which is fully paralyzed by the drug.

On the other hand, the evidence of thymic relationship to myasthenia gravis seems to be accumulating. Dr. Eaton's analysis appeared to

* Paper not included in this issue.

confirm the observations of the Boston group that thymectomy in young women exerts a favorable influence as compared with those in control groups. What this will ultimately mean in terms of therapy and understanding of the disease remains to be seen. Sceptics like Grob are still unwilling to concede that thymectomy is any more than a continuing experiment. However, the evidence as it accumulates seems to favor the point of view that thymectomy may influence the disease.

The next section constituted an examination of endocrine factors other than thymus in this disease. These papers were listed to review the effect of endocrine changes in pregnancy and in work, as well as the influence of endocrine diseases and administration of hormones upon myasthenia gravis. In pregnant women who have this disease an important observation is the fact that certain of the babies of myasthenic subjects have myasthenia gravis (presumably transmitted from the mother) which clears up in a short time. As to the effect of pregnancy on the disease in the mother, the data presented by Drs. Schlesinger, Osserman and others shows that a third of the patients became worse, a third remained unchanged and a third improved. Although these figures seem to provide no direct relationship, no doubt some subjects improve remarkably during pregnancy, usually in the latter part, and relapse after delivery. For these patients one does not like to abandon the possibility that there might be an association.

Dr. Ingle discussed the endocrine compounds (emphasizing the adrenal cortical extracts) which favorably influence the work performance of the normal rat and described the favorable action of posterior pituitary hormones. Dr. Kane gave an excellent review of the harried subject of endocrinopathies in relation to the myasthenic process. In this paper opinion seemed to crystallize that ACTH has an adverse effect upon this disease, despite contrary views previously published. Although he emphasized that improvement in myasthenia occurs from thyroid therapy more often than the reverse, many have felt that hyperthyroidism unfavorably influences the disease. The association here again is puzzling in that it is not direct.

In the section on drug therapy two new compounds were discussed. The advantages and the disadvantages of their longer action and of the lesser parasympathetic effect were noted. The so-called cholinergic crisis produced by these and other drugs is a very real problem. Many persons have thrown themselves into this serious condition by inadequately supervised self-medication.

As Dr. Viets has stated, the mortality rate of myasthenia has been reduced to about 10 to 15 per cent by modern therapy, yet as far as we know these drugs are symptomatic therapy and do not influence the disease process directly. They are not in any way concerned with development of remissions. To me it seems that the therapy of the future should be concerned with the possibility of inducing a remission rather than treating the established disability. Fatalities continue to occur, and a more definitive method of treating this disease is needed. It must be pointed out that should we discover, as a cause of the defect in myasthenia, a circulating toxin, our problem may not thereby be solved. One needs only to recall that the cause of botulism is known, yet no therapy is effective. The same might be said of tetanus. Both of these conditions have a resemblance, although in opposite directions, to the myasthenic process.

GEORGE D. GAMMON, M.D.
University of Pennsylvania Medical School
Philadelphia, Pennsylvania

Symposium on Myasthenia Gravis

Problems of Myasthenia Gravis

HENRY R. VIETS, M.D.
Boston, Massachusetts

THE problems surrounding myasthenia gravis are multiple and diverse. Many questions posed by the disease cannot be answered with any degree of confidence at the present time. Some, particularly those of a clinical nature, have been resolved by the discriminating observations of patients by previous workers, for the disease has been adequately recognized since Erb's description in 1879 and Goldflam's in 1893; Jolly's nomenclature was suggested in 1895 and generally accepted after 1899. Most of the clinical facts were known by 1900 and Weigert, a year later, had even disclosed a thymoma in one of Laquer's patients at autopsy.

Willis had of course grasped the one essential sign as early as 1672, namely, the faulty and impaired weakness of voluntary muscles evoked only by repeated stimulation with a tendency toward recovery upon rest. Thus the disease having only one sign was relatively easily diagnosed by our predecessors, even as it is today in most cases. By 1900 much of the symptomatology was recognized: age, sex, sudden onset after emotional or physical trauma, and variations at puberty and during the puerperal state. Localization with common muscular groupings relative to cranial nerve innervation, neck weakness, and weakness of extremities and back, had been fully described. From 1900 to 1934 the disease was considered uncommon and usually fatal. No pathologic changes were noted in the nervous system and only the thymic abnormality and "lymphorrhages" in the skeletal muscles were noted. The stimuli which led to our present day concept for myasthenia gravis came largely through the discovery of the junctional transmission of nervous effects by chemical agents by Otto Loewi (1921), Henry H. Dale (1933) and others, the striking response of physostigmine and later neostigmine in overcoming the muscular deficiency in patients by Mary B. Walker (1934) and the suggested relationship between the thymus and myasthenia gravis by Blalock, Keynes, Andrew Wilson and others (1941 to date). These and other contemporary investigations have revived an interest in the disease (which had nearly died out after the turn of the century) and led to the establishment of a Myasthenia Gravis Foundation. The calling of this Conference in 1954 was for the purpose of exchanging ideas on the problems presented by twenty years of research since Walker's brief letter to the editor of the *Lancet* was published on June 2, 1934. Few physicians in the history of medicine have been more privileged than Mary Walker in establishing a new trend in medicine, accomplished by acute clinical observation at St. Alfege's Hospital in the Spring of 1934. This Congress and indeed the whole myasthenia gravis awakening owes a debt to her; by meeting here today we are attempting to repay a debt, long overdue.

I would like to take this opportunity to discuss briefly three main problems, each presenting fundamental questions of importance to our understanding of myasthenia gravis.

THE PROBLEM OF JUNCTIONAL TRANSMISSION

Prior to Loewi's classical demonstration of the release by vagus and sympathetic impulses of substances resembling acetylcholine and adrenaline, T. R. Elliott had made such a suggestion as early as 1904. It was not until 1929, however, that Dudley and Dale were able to recognize acetylcholine as a natural body constituent and later suggest that peripheral nerve impulses liberated this substance at the synapse. Although no one has been able to collect a sufficient quantity of acetylcholine transmitting the effects of nerve impulses at the endplate area to be able to identify it chemically, acetylcholine is a very unstable choline ester, rapidly destroyed by cholinesterase and synthesized by cholineacetylase. However, the transmitter may be concentrated at the nerve ending together with the

specific enzymes for its formation and its destruction; the chemical may also be found along the nerve fiber and perhaps even at the cell of origin. The system is not specific for the nerve ending alone. It cannot be assumed that acetylcholine must act as a stimulator for all cells or fibers from which it can be extracted. The problem needs further elucidation, but in myasthenia gravis at least it would seem that the muscle at its receptive area in contact with a cholinergic fiber end is sensitive to acetylcholine or, as Dale put it, "cholinoceptive." It is possible that cholinergic function and cholinoceptive sensitiveness are different characters which need not, and often do not, go together.

We need to know where the acetylcholine and its enzymes originate. Do they come from the nerve cell and pass down the fiber to maintain a physiologically effective depot at the ending? These and many other problems await the answers from neurophysiologists, the final interpretation of which may profoundly affect our concept of what happens in myasthenia gravis when the cholinesterase is inhibited by neostigmine and the system, at least for the time being, is set right and function is restored. We are far from our goal. Can we be certain that all nerve impulses are released by chemical transmitters and, if they are, can all the transmitters be identified? We need further information regarding one-way restriction, the effects of poisons and the part played by many drugs influencing this system of nerve transmission. Much is in the realm of pharmacology, aided by the electrical and biophysical methods of physiology. To these tasks a research program for the Myasthenia Gravis Foundation should in part be devoted. Although the chance clinical observation of Mary Walker has resulted in prolonging many lives and making possible a wider study of the disease and its course in man, we can not hope for many inspirational flashes of a similar nature for, welcome as they are, medical advance fundamentally depends on well planned experimentation without limit of time or substance.

THE THYMUS AND MYASTHENIA GRAVIS

That the thymus bears some relationship to myasthenia gravis is now widely accepted. What the relationship is leads us at once into a field of physiologic theory and clinical speculation. The evidence for thymic involvement rests on the pathologic findings of thymic change in a large percentage of patients with myasthenia, the favorable results of thymectomy in selected cases of the disease, the formation of thymomas in about 15 per cent of cases with thymic abnormalities and the experimental evidence of Wilson which revealed that an extract of the thymic tissue removed by thymectomy appears to depress contractions in muscle-nerve preparations to a degree comparable to that produced by tubocurarine. Thus writes Keynes on the "physiology of the thymus gland," and Levitt went so far as to refer to "thymic myasthenia." Many would feel, with considerable justification, that conclusions of this type have not been established to the point of acceptance. There is still need for continued investigation, and profit may be expected to result from extended studies of postoperative patients, particularly in selecting those most likely to be improved by thymectomy and in evaluations of experimental extracts along the lines suggested by Wilson and others. Does the thymus play a substantial or even a subsidiary function in myasthenia gravis? Is it subordinate to the pituitary or others in the endocrinological system? One can hardly conceive of such a prominent structure, so profoundly affected in myasthenia gravis, as having no relationship to the disease. Thymic studies are of primary importance in expanding our knowledge of the disease. Such a promising field should not be neglected.

THE PROBLEM OF MEDICAL CARE

Shortly after 1934 neostigmine (prostigmin) became the standard form of treatment for patients with myasthenia gravis. In 1954 it still holds a primary place in overcoming the muscular deficiency for it has shown no tendency toward habit formation, it rarely becomes ineffective because of tolerance and has maintained its effectiveness throughout the years; it is relatively easily adjusted to suit the patient's needs and its muscarine-like stimulation of the parasympathetic nerves is usually readily controlled by atropine. On the other hand, the drug has the defect of being short-acting, the usual response from oral medication being one to three hours and, when given intramuscularly or intravenously, much shorter. Thus many patients require some medication every two hours during the day and every three or four hours during the night. Many drugs have been given a trial during the last twenty years, including other anticholinesterase preparations, but

none have been found as useful over a long period of time as neostigmine. As the use of the drug in myasthenia gravis is virtually one of replacement therapy, only that medication will be satisfactory that can be taken with safety and results in a minimum discomfort from overdosage or underdosage. Recently pyridostigmine (mestinon®) has shown a tendency to replace neostigmine, but the search for a better and longer lasting drug must continue, and this Conference may stimulate research along these lines. Our knowledge of neostigmine is still lacking for we do not know where it is absorbed in the gastrointestinal tract, how it is eliminated, where it is stored in the body and what blood level is obtained. It is not known when or why some patients with myasthenia gravis can apparently use to advantage 1,500 mg. or more of the drug in a day, whereas 15 or 30 mg. is not easily tolerated by normal persons. These and other similar problems call for answers before our therapy can be improved and before we can reach a goal of full and continued maintenance of muscular power.

In posing three major problems, many others will occur to all of us. The basic cause of the disease still eludes us, as do the reasons for its onset at various age levels, the cause of relapse and remission progression, the transmission of the symptoms to the child by the pregnant mother with the disease, the reasons for its variable location in particular muscle groups and, finally, the problem of atypical cases or those showing poor neostigmine response need further elucidation. Such problems set a pattern for future research.

Structure of the Motor Endplate*

GEORGE B. KOELLE

Philadelphia, Pennsylvania

In his comprehensive review on the innervation of skeletal muscle, Tiegs[1] quotes the statement made by Kuffler[2] that "the end plate has become, in recent years, a physiological entity which at present cannot be strictly identified with the structure of the histologists." One reason for this hiatus in our knowledge is the disagreement which exists among histologists concerning the embryologic derivation and the limiting boundaries of some of the structures which comprise the endplate. The controversial points are discussed in detail in Tiegs' review; the present description is drawn chiefly from the pioneer work of Kühne[3] and the more recent studies of Couteaux[4,5] and Young[6] and their associates.

Centrally to its point of contact with the muscle fiber, the motor axon is ensheathed in a layer of myelin which is closely invested by the Schwann cells. The latter are surrounded by a continuous sheath called the neurilemma external to which is the sheath of Henle, a continuation of the perineural sheath surrounding the entire bundle of nerve fibers. Near the endplate region the myelin sheath is lost, and the sheath of Henle becomes continuous with the outer sarcolemma, an extraneous sheath derived from the endomysium. According to Couteaux,[4] at the endplate the axon retains a surrounding sheath, the teloglia, derived from the sheath of Schwann, and remains external to the true sarcolemma. The latter is modified, beneath the axon with its investing teloglia, as the pallisade-like subneural apparatus. Whether the neurilemma or a derivative is retained is less certain. Surrounding the subneural apparatus is a fairly well circumscribed region of granular sarcoplasm which is rich in nuclei. The entire motor endplate occupies a third or less of the circumference of the muscle fiber.

Couteaux has focussed attention on the subneural apparatus as the probable site of both the acetylcholine(ACh)-receptor substance, where the endplate potential is initiated, and most of the acetylcholinesterase (AChE) of the endplate. The subneural apparatus was demonstrated initially by selective staining with Janus Green B, and was found to have a lamella-like structure. The same pattern is obtained when the endplate is stained for AChE[5] by the acetylthiocholine method,[7,8] whereas staining for the enzyme is considerably lighter in the axon and its terminal arborization. Thus in accordance with earlier deductions[9,10] most of the AChE at the endplate is postsynaptic and proportionately little is presynaptic. This is the reverse of the situation present in the majority of the synapses in sympathetic ganglions[8,11] and may account for some of the physiologic and pharmacologic differences between neuromuscular and ganglionic transmission.

Couteaux's depiction of the interposition of the teloglial sheath between the axon and the subneural apparatus is analogous to the situation in autonomic ganglia described by de Castro.[12] According to the latter author the preganglionic fibers are ensheathed in glial cells and thus do not make direct contact with the ganglionic neurons or their dendrites. If both conceptions are correct the influence of the glial elements on ganglionic and neuromuscular transmission under normal and pathologic conditions must be considered. The ganglionic glial cells and the Schwann sheath cells, from which the former take their origin, contain non-specific or pseudo-ChE, the function of which remains to be determined. The suggestion that the enzyme is responsible for maintenance of the myelin sheath[13,14] has been disputed.[15] It is quite possible that the low concentration of non-specific ChE recently demonstrated by Denz[16] at the motor endplate is localized in the Schwann cell–derived teloglia.

Until recently, histochemical findings indi-

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cated that the AChE of the nervous system was present only in cholinergic neurons, including those giving rise to motor fibers, preganglionic fibers and cholinergic postganglionic fibers, and certain neurons of the central nervous system. By the use of a selective inhibitor* of AChE to rule out staining due to hydrolysis of the histochemical substrate (acetylthiocholine) by other enzymes, and by employing a prolonged incubation period (two hours), it has now been found that adrenergic and sensory neurons contain relatively low concentrations of AChE.[17] This requires modification of the previously expressed conclusion[8] that histochemical evidence is wholly incompatible with Nachmansohn's[18] theory of the role of acetylcholine in the conduction of nerve impulses, the general aspects of which have been discussed by Eccles,[19] Gerard[20] and Bullock.[21] The significance of the small amounts of the enzyme present in adrenergic and sensory neurons, the acetylcholine and choline acetylase concentrations of which are also extremely low, has not been established.

Although there have been reports of nonspecific pathologic changes in the muscle fibers of patients with myasthenia gravis,[22] there is practically no information available concerning possible alterations in the details of the structures described. Studies employing specialized histologic and histochemic technics should be undertaken, in conjunction with physiologic and biochemic investigations, in future attempts to elucidate the nature of this disease and thereby advance the therapy.

* 1-5-bis(4-allyl dimethylammonium phenyl) pentan-3-one dibromide or B.W. 284c51.

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Neuromuscular Transmission*

Fundamental Aspects of the Normal Process

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In the vertebrate animal the normal neuromuscular transmission process involves the following sequence of events: nerve action potential → acetylcholine release → combination of acetylcholine with endplate membrane receptor → decrease in transmembrane potential at the endplate → muscle action potential → muscle contraction. These steps are considered from the biophysical point of view in two recent reviews.[1,2] In this article discussion is limited to a few selected topics chosen to illustrate certain principles and to point out some modern research technics now used in the study of neuromuscular transmission.

Initiation of Contraction; Muscle Cell Membrane Potentials. We may begin with the last step of the transmission process, namely, shortening of the contractile system in the intact muscle cell subsequent to the propagation of a membrane action potential along the length of the fiber. At present the nature of the coupling between the membrane action potential and activation of the contractile system is unknown. The muscle cell transmembrane potential characteristic of the resting and active states may be recorded by piercing the fiber with a fine microelectrode.[3] A typical record so obtained is shown and explained in Figure 1.

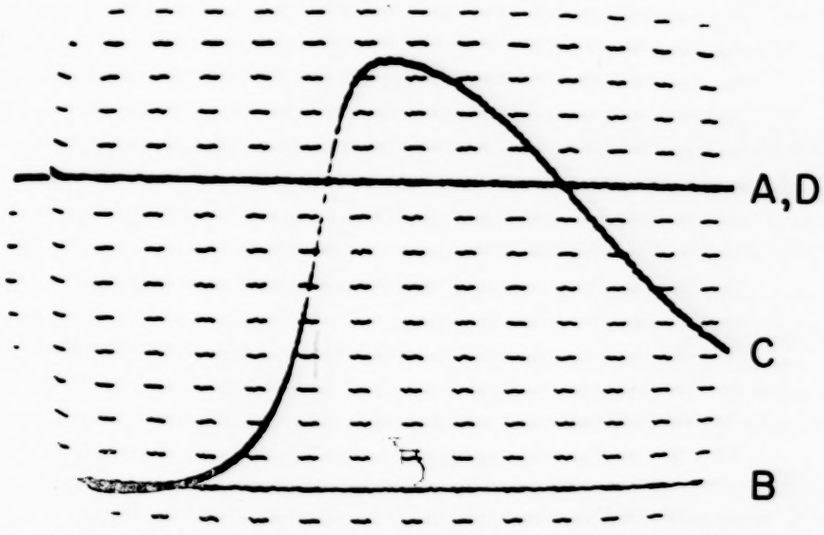

FIG. 1. A typical resting and action potential record obtained distant from the endplate. A and D are records with the microelectrode outside the fiber at the beginning and end of the experiment. B and C are records with the microelectrode inside the fiber. In B the fiber is at rest. C shows the activity which results from stimulation of the motor nerve. Calibration grid; ordinate 10 mv. steps, abscissa 0.2 millisecond intervals. The last portion of the falling phase was not recorded.

Initiation of the Muscle Action Potential. A propagated muscle action potential may be initiated at that point along the length of a muscle fiber where the resting membrane potential is suddenly reduced to a critical value. If the membrane potential does not reach the critical

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level, the disturbance remains localized and the membrane potential returns to the resting level, no propagated action potential being elicited. These facts are illustrated in Figure 2 which shows the membrane potential of a single muscle fiber recorded at the zone of application of a short electrical current of variable intensity.

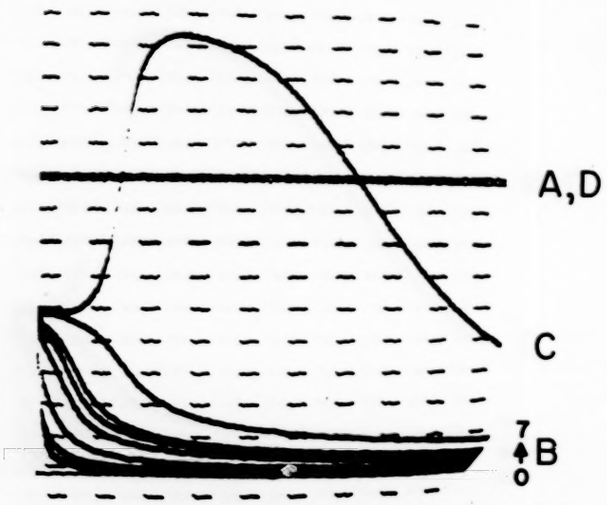

FIG. 2. Reduction in membrane potential recorded in the zone of application of an outwardly directed electric current. A,D and calibration grid as in Fig. 1. B_{1-7}, series of records showing return of the membrane potential toward the resting level of −92 mv. (B_0) immediately after the extrinsic electric current is removed. In B_1 current strength is least, in B_7 greatest. C is a propagated action potential produced by applying current slightly stronger than in B_7. Critical membrane potential at which a propagated action potential is produced is −41 mv.

It can be seen that at the close of a weak stimulus the membrane potential returned to the resting value. A stimulus strong enough to reduce the membrane potential from 92 to 41 mv. produced a critical state whereby a propagated action potential arose.

Changes in membrane potential of a muscle fiber at the endplate occurring during neurogenic initiation of an action potential have been studied using an internal microelectrode.[4] A typical record obtained thereby[4] is shown in Figure 3. Similar results have been reported by

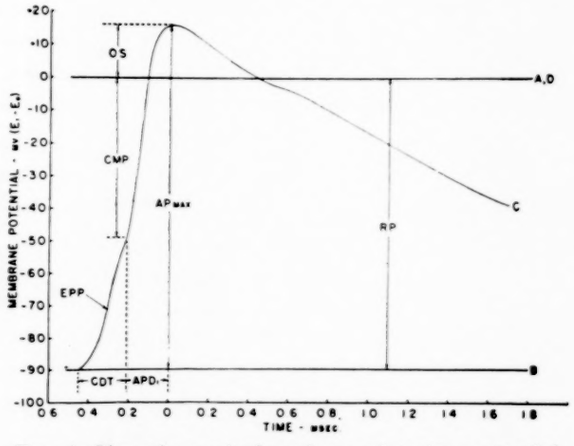

FIG. 4. Plot of a typical resting and action potential record obtained at the center of the endplate to illustrate some of the nomenclature used in this paper. A, B, C and D as in Figure 1. EPP, endplate potential; CMP, critical membrane potential. For further details see reference 4.

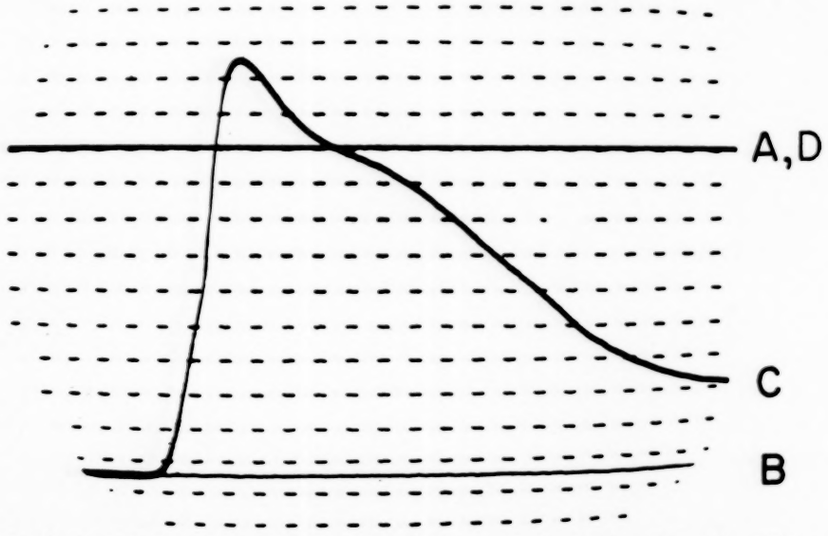

FIG. 3. A typical resting and action potential record obtained at the center of the endplate. A, B, C, D and calibration grid as in Figure 1. Trace C was produced by stimulating the motor nerve. (Rising phase of trace C retouched.)

Fatt and Katz.[5] For *in vivo* neurogenic initiation of muscle action potentials at the endplate, like the electrical initiation just described, it is required that the transmembrane potential be reduced to a critical value.

For descriptive purposes, a typical membrane action potential recorded at the endplate is redrawn in Figure 4. When a nerve action potential arrives, it causes localized reduction of membrane potential at the endplate called the endplate potential (EPP). This record shows that the membrane potential was reduced to the critical level (CMP), and as a consequence a propagated action potential was initiated in the muscle fiber.

As is well known, the principal action of *d*-tubocurarine is to reduce the sensitivity of the endplate to acetylcholine. Therefore if sufficient *d*-tubocurarine is applied to a nerve-muscle preparation, neuromuscular transmission may be blocked, i.e., a nerve action potential will no longer initiate a muscle action potential. Under these conditions, with the intracellular microelectrode at the endplate, an uncomplicated endplate potential is recorded, as shown in Figure 5. These records were obtained by stimulating the nerve at three-second intervals, during which time the endplate was perfused with a minute volume of Ringer's solution in order to remove *d*-tubocurarine.[6] The result was a very prompt increase in the amplitude of the EPP until finally the CMP was reached and thereby a propagated action potential was initiated. Washout of *d*-tubocurarine caused no change in the time course of the EPP, indicating that its removal simply increases the sensitivity of the endplate membrane for acetylcholine. If the washout of *d*-tubocurarine is continued, the EPP rises with greater speed, the CMP is reached earlier and the result is a decrease in transmission delay at the n-m junction.

Membrane Potential Changes Produced by Transient Application of Acetylcholine to the External Surface of the Endplate Membrane. Acetylcholine is thought to react with the endplate membrane causing this structure to increase its permeability to ions. Further information on this point can be gained from studying the reaction under conditions where the extracellular electrolyte composition is varied. Unfortunately, variations in electrolyte usually affect the behavior of the nerve and therefore in such experiments it is necessary that some other means be used to apply small constant amounts of acetylcholine to the endplate. The latter problem has been solved at least partially by development of a method in which acetylcholine is applied electrophoretically.[7] A brief description of the method is as follows: The membrane potential of the muscle cell at the endplate is recorded with an internal microelectrode. (Fig. 6.) At a point 10 to 20 μ from the recording site, acetylcholine ions are delivered electrophoretically to the external surface of the endplate. To do this a microneedle is filled with acetylcholine (0.55 M) and contact with the solution is made via an Ag-AgCl electrode. Diffusion of acetylcholine ions is prevented by applying a negative potential to the electrode. Brief ejection of acetylcholine ions occurs when the electrode potential is driven positive by applying a 5 millisecond positive square wave voltage pulse to the system as indicated.

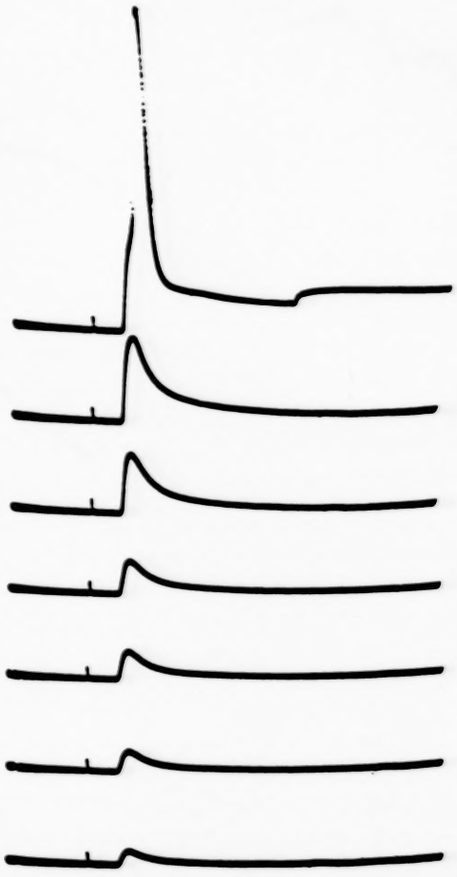

FIG. 5. Serial group of endplate potential records obtained at 3 second intervals during perfusion of a muscle fiber immersed in Ringer's solution plus *d*-tubocurarine, 5.3 mg./L. Perfusion with Ringer's solution began with the record at the bottom.

When increasing amounts of acetylcholine ions are applied to the endplate by this method, the membrane potential at this site is progressively reduced, as shown in Figure 7. "Endplate potentials" of increasing magnitude are produced until finally the critical membrane potential is reached and a propagated action potential is elicited. If the amount of applied acetylcholine ions is increased still further, multiple action potentials appear.

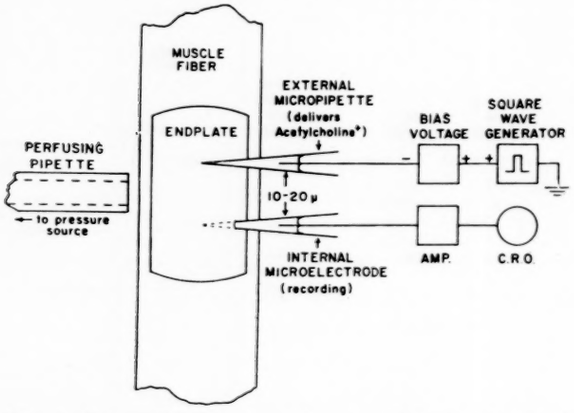

FIG. 6. Schematic diagram showing the experimental arrangement used when recording changes in the transmembrane potential produced during microelectrophoretic application of acetylcholine ions to the outer surface of the endplate. The pipette to the left is used when changing the composition of the extracellular fluid in this zone. For further details see text.

Mechanism by Which Acetylcholine Reduces the Membrane Potential at the Endplate. Any change in membrane potential means that the distribution of electric charge (ions) across the membrane is altered. Reduction of the membrane potential requires ionic redistribution, as stated in Figure 8.

It follows that acetylcholine facilitates transmembranal movement of ions, a process alternatively described as an increase in membrane permeability. A likely source of inward moving positive charge would be Na^+ ions, that is to say, one action of acetylcholine might be to increase the Na^+ ion permeability of the end plate membrane. To test this hypothesis it is necessary to alter the extracellular Na^+ concentration and then measure the change in membrane potential during high speed transient application of a constant amount of acetylcholine to the outer surface of the endplate membrane, using the electrophoretic method.

Two types of experiments were performed.[8] In the first type (Fig. 9) the muscle was placed in normal Ringer's solution and single endplates were perfused with NaCl-free Ringer's solution. Sucrose was substituted to maintain constant osmotic pressure. Before, during and after the perfusion the membrane potential change produced subsequent to electrophoretic application of a small quantity of acetylcholine ions was recorded. It can be seen that removal of NaCl virtually abolishes the response to acetylcholine ions.

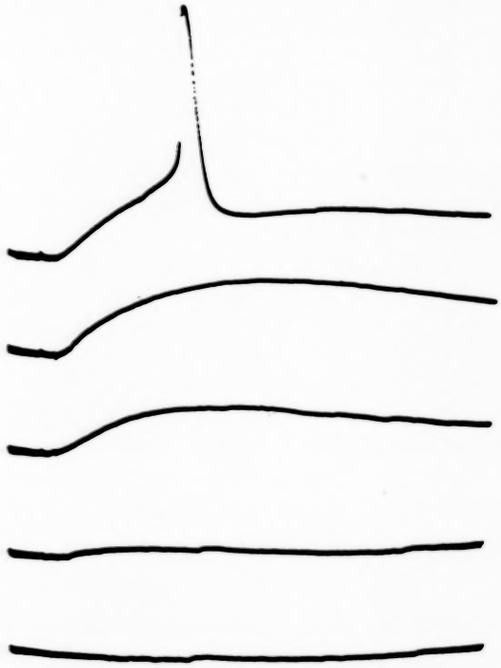

FIG. 7. Endplate potentials produced by microelectrophoretic application of acetylcholine ions to the endplate in successively increased amounts. Intracellular recording.

The second type of experiment (Fig. 10) was essentially like the first except that the muscle was equilibrated in Ringer's solution containing no NaCl and the endplate was perfused with normal Ringer's solution It can be seen that this perfusion restores the response to application of acetylcholine ions. From a series of such experiments it was found that the response of the end-plate membrane to acetylcholine ions is reduced at least by a factor of 14 when extracellular NaCl is removed.

The conclusions one may draw from the preceding experiments are: (1) combination of acetylcholine ions with endplate receptors is inhibited when the total ionic strength of the extracellular solution is reduced; (2) a prominent response of the endplate membrane when

reacted with acetylcholine ions is an increase in its permeability to Na^+ ions.

At present there is no evidence bearing on the validity of the first possible conclusion. This is a critical point which merits further investigation. It must also be pointed out that the increase in average, 5×10^{-16} equivalents of acetylcholine ions are applied to the endplate.[7] Since the application is a wasteful process, the minimum amount of acetylcholine ions required must be even smaller. Fatt and Katz[5] have shown that the net charge transported across the

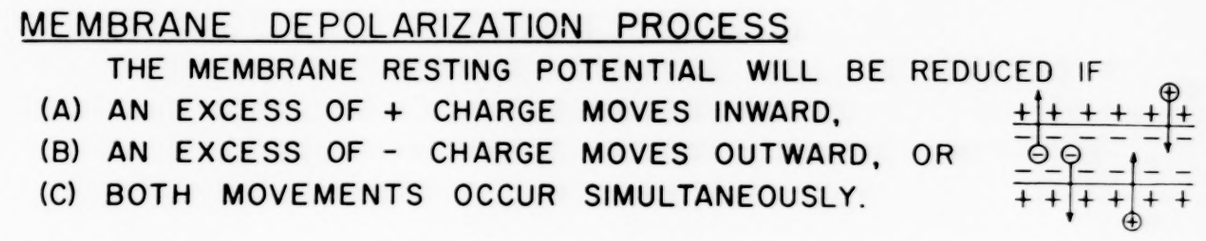

FIG. 8. Membrane depolarization process.

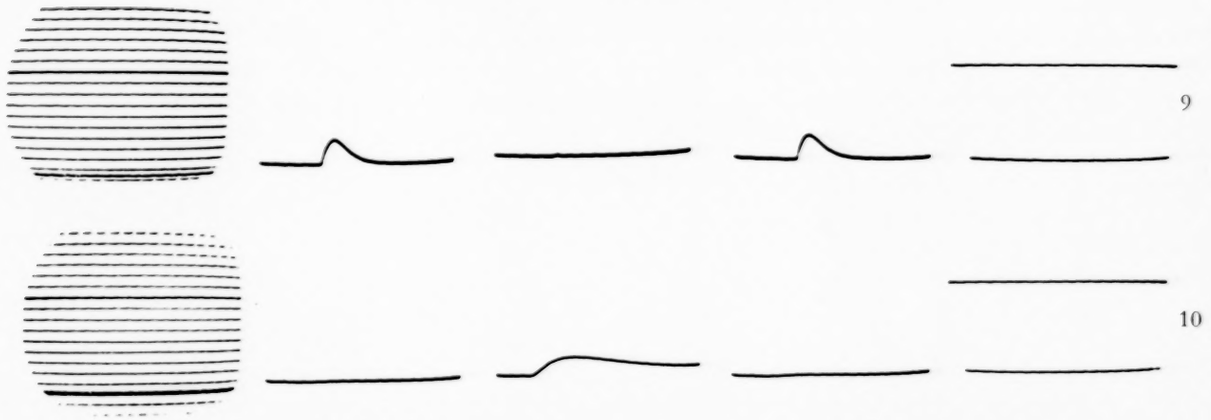

FIG. 9. Typical endplate potential changes produced by microelectrophoretic application of acetylcholine ions to the endplate of a single muscle fiber. The fiber was immersed in normal Ringer's solution and perfused with "sucrose Ringer's" solution (NaCl-free). Peak amplitudes of the endplate potentials were as follows: before perfusion 24 mv., during perfusion less than 1 mv., after perfusion 23 mv. Resting potentials measured during these periods were 90, 89 and 90 mv.

FIG. 10. Typical endplate potential changes produced by microelectrophoretic application of acetylcholine ions to the endplate of a single muscle fiber. The fiber was immersed in "sucrose Ringer's" solution (NaCl-free) and perfused with normal Ringer's solution. Peak amplitudes of the endplate potentials were as follows: before perfusion 1 mv., during perfusion 18 mv., after perfusion 2 mv. Resting potentials measured during these periods were 87, 80 and 86 mv.

permeability brought on by acetylcholine ions is not selectively limited to Na^+ ions but appears to include the K^+ ion as well. For further evidence on this matter see references 2, 5 and 9.

"*Trigger Function*" *of Acetylcholine.* As stated previously, the genesis of the endplate potential depends at least in part on the penetration of the membrane by positively charged extracellular ions. Released acetylcholine ions might act as a source of penetrating positive charge but it is a fact that the total electrical charge which moves is much too great to be entirely accounted for in this way. To illustrate, by means of microelectrophoresis it can be shown that a propagated action potential may be elicited if, on the membrane during a subthreshold endplate potential is 80×10^{-16} equivalents, and in the absence of *d*-tubocurarine the figure increases to 200 to 400×10^{-16} equivalents. Hence, each acetylcholine ion facilitates transmembranal movement of at least 20 to 75 additional univalent ions and in this sense it may be thought of as having a "trigger function."

Release of Acetylcholine. The mechanism by which acetylcholine ions are released during the "resting" state and subsequent to arrival of a motor nerve impulse has been the subject of intensive investigation by Katz and his collaborators. This important work cannot be discussed here and the reader is referred to the

reviews of Fatt,[1] and Castillo and Katz[2] which deal more extensively with the subject.

Acknowledgment: The work of this laboratory was aided by a grant from Hoffmann-La Roche, Inc, Nutley, N. J.

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Neuromuscular Blocking Agents*

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Since 1948 the investigation of neuromuscular blocking agents has been in a turmoil. The careful studies of Paton and Zaimis[1] on decamethonium and homologues showed that the paralysis produced by these agents was different from the paralysis produced by tubocurarine. Even though the blockade produced by decamethonium was limited to the neuromuscular junction, it was found that neostigmine did not antagonize the block; in fact in some instances it would make it worse. Burns and Paton[2] observed that the blockade produced by decamethonium coincided with a sustained depolarization of the endplate region. Since tubocurarine does not produce such a depolarization, we can classify neuromuscular blocking agents as *depolarizing neuromuscular blocking agents* and *non-depolarizing neuromuscular blocking agents*. Many statements have appeared in the literature that substances are depolarizing neuromuscular blocking agents without direct evidence of depolarization of the endplate region. The indirect evidence is based on effects such as spastic paralysis in the pigeon, contracture of the rectus abdominis of the frog, lack of antagonistic effect with neostigmine, and the height of contraction during and after tetanic stimulation. All these are useful tools, but the ultimate classification, whether an agent blocks by depolarizing or without depolarizing, lies in recording the depolarization and comparing this with the observed neuromuscular blockade. In our laboratory we used, slightly modified, the technic described by Burns and Paton.[2] A moving electrode scans the surface of a muscle and the potential between the moving electrode and a stationary electrode placed near the tendon of the same muscle is recorded. The results lead to the following classifications: (1) Depolarizing neuromuscular blocking agents—decamethonium, succinylcholine, succinylmonocholine, neostigmine and edrophonium (tensilon®); (2) Non-depolarizing neuromuscular blocking agents—tubocurarine, dimethyl-tubocurarine, flaxedil,® mytolon® and 1 : 5 di (N-allyl-N-di-methylaminophenyl)pentan-3-one(53–67).

In the list of depolarizing neuromuscular blocking agents succinylmonocholine is of interest because it is the first degradation product of succinylcholine[3] and is still an effective depolarizing neuromuscular blocking agent, a fact which should not be overlooked by those who use succinylcholine.

Neostigmine, edrophonium and (53–67) are primarily cholinesterase inhibitors and as such are antagonists to tubocurarine. In larger doses all three can produce neuromuscular blockade. With neostigmine and edrophonium this blockade is accompanied by a sustained depolarization whereas with (53–67) no such depolarization is evident.

Zaimis[4] compared the paralyzing dose of decamethonium and of tubocurarine in different species. If the species were arranged so that the paralyzing dose of decamethonium increased when going toward the right, the paralyzing dose of tubocurarine tended to decrease in that same direction. When we compare different muscles of the same species, e.g., the soleus and tibialis of the cat, we find that the paralyzing dose of decamethonium is smaller for the tibialis than for the soleus whereas with tubocurarine the opposite is true.[5] In Figure 1 an attempt is made to explain the different behavior of the soleus and tibialis muscles. Although it is oversimplified, this simple hypothetic graph helped me very much in tying controversial facts together. The distance from top to bottom represents the membrane potential. The dashed line arbitrarily placed in the middle represents the critical membrane potential. This is the membrane potential at which, after quick depolarization, an action potential ensues. I should like to assume that a sustained depolarization at this level will produce blockade.

After stimulation of the sciatic nerve acetyl-

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choline is released at the endplate region and, according to our present concepts, this acetylcholine gives rise to an endplate potential (e.p.p.). In Figure 1 I propose that the resulting e.p.p. is different in the two muscles. An important factor may be the relative relation between the e.p.p.'s. In other words, the sustained level of depolarization produced by a certain dose of decamethonium is lower in the soleus than in the tibialis. In the tibialis the sustained depolarization would reach the critical membrane potential and thus no action potential or twitch could

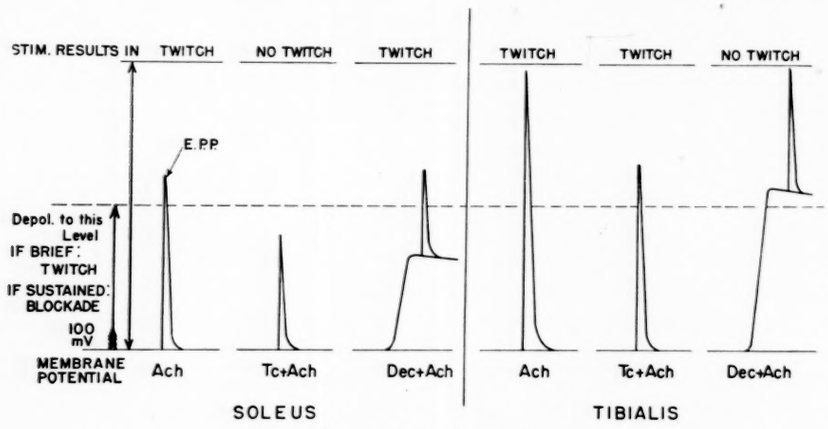

FIG. 1. For explanation see text.

the heights of the e.p.p. and the critical membrane potential; this is shown in Figure 1. Another important factor may be the angle at which the rising phase of the e.p.p. reaches the critical membrane potential.

What happens after the administration of tubocurarine? The competitive antagonism between acetylcholine and tubocurarine will prevent a certain portion of the acetylcholine from reacting with the receptive substance and the result is a smaller e.p.p. Let us assume that the e.p.p. is decreased to two-thirds its original height. In the soleus muscle this depolarization is, according to our hypothetic scheme, not enough to reach the critical membrane potential and thus tubocurarine prevents the production of an action potential or twitch. In the tibialis, however, two-thirds the original e.p.p. still reaches the critical membrane potential and thus the action potential or twitch is still present. In order to produce paralysis of the tibialis we have to increase the dose of tubocurarine so that the e.p.p. may be diminished to half its original height. In other words, as far as tubocurarine is concerned the paralyzing dose for the tibialis is higher than that for the soleus.

What about decamethonium? Here the sustained level of depolarization is important. Let us now assume that the ratio of the depolarization produced by decamethonium in the two muscles is the same as the ratio of the heights of occur. In the soleus, on top of the sustained depolarization, the e.p.p., even though it is diminished, reaches the critical membrane potential; and since this is a quick depolarization, we will observe a normal twitch. In order to produce paralysis with decamethonium in the soleus we have to increase the dosage. Thus, the paralyzing dose of decamethonium for the soleus is higher than that for the tibialis.

The e.p.p.'s after decamethonium are drawn smaller than the original e.p.p.'s because I believe that besides the depolarization produced by decamethonium there exists also a mutual antagonism between acetylcholine and decamethonium similar to that proposed for tubocurarine.

We observed differences between decamethonium and tubocurarine. Are there similarities in their effects?

Frequency of Stimulation. Increased frequency of stimulation of the sciatic-gastrocnemius preparation of the rat results in a decrease of the paralyzing dose for both depolarizing and nondepolarizing neuromuscular blocking agents.[6]

Neostigmine Antagonism. (1) In the isolated lumbrical muscle of the rabbit Jenden et al.[7] described two phases of paralysis after the administration of decamethonium. The first phase has a rapid onset, is short-lasting and cannot be antagonized by neostigmine. This is the usual blockade seen after decamethonium in

contrast to the second phase. The second phase is slow in onset, outlasts the first phase and can be antagonized by neostigmine. (2) In the intact cat Jewell et al.[8] found that after two or three injections of decamethonium the neuromuscular blockade in the soleus can be antagonized by neostigmine. (3) In some myasthenia gravis patients Churchill-Davidson et al.[9] observed that paralysis produced by decamethonium was antagonized by neostigmine.

In terms of Figure 1 all these facts can be explained if we suppose that the safety factor, i.e., the difference between the e.p.p. and the critical membrane potential, is diminished. This could be caused by a lack of free acetylcholine, a lack of free receptive substance, an inability of the endplate region to undergo adequate depolarization or a change of the critical membrane potential. In more general terms, we can try to formulate what occurs at the endplate with the aid of Figure 2. I have assumed a reversible reaction between acetylcholine and the receptive substance which leads to the formation of AchR. The amount of AchR produced depends on the concentration of the free receptive substance and the free acetylcholine. Also, AchR is responsible for the local depolarization. When Ach comes in a single burst from the nerve impulse, AchR is short-lived and results in a quick depolarization and a quick repolarization, better known as the endplate potential. Under special circumstances it is possible to maintain a large amount of AchR at the endplate. The result then is a sustained depolarization which in itself produces neuromuscular blockade similar to the one seen after administration of decamethonium.

Tubocurarine and acetylcholine are both assumed to compete for the same free receptive substance. Therefore in the presence of tubocurarine the acetylcholine released after a nerve impulse will find a smaller concentration of free receptive substance. The result is that less AchR is formed and there is therefore less depolarization at the endplate. The combination of tubocurarine and receptive substance does not lead to depolarization, and hence tubocurarine produces its block by decreasing the total amount of free "R" available for acetylcholine.

With decamethonium the picture is slightly more complex. Let us assume that decamethonium reacts with the receptive substance in the same way as does acetylcholine. It will therefore occupy a certain amount of the free receptive substance, leaving a smaller amount available to react with acetylcholine. DecR, however, by itself produces a depolarization of the endplate. Under special circumstances this depolarization produced by DecR is quick enough to result in a twitch. DecR is ordinarily long-lasting (that is, more than one minute)

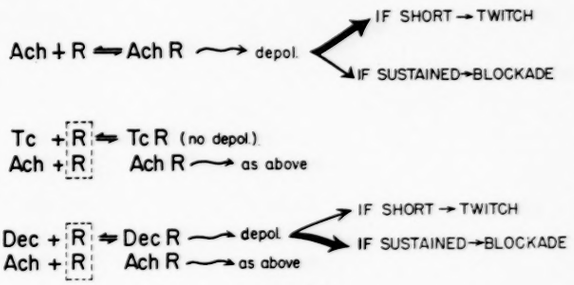

FIG. 2.

and the sustained depolarization which it produces is the primary cause of neuromuscular blockade seen after administration of decamethonium. One can, however, theorize that at certain occasions or in special motor endplates the sustained depolarization produced by DecR is not the primary cause of blockade. If motor endplates exist in which the depolarization from the maximal amount of AchR after maximal stimulation is barely able to reach the critical membrane potential, then one may postulate that in these same muscles the concentration of free receptive substance is a very important factor for neuromuscular transmission. Thus, these muscles will be very sensitive to tubocurarine. Furthermore, if the maximal amount of AchR is relatively ineffective in these muscles in producing depolarization adequate to stimulate, we can assume that DecR is equally ineffective in producing the sustained depolarization necessary to block. Therefore, it will require a large amount of decamethonium to produce a depolarizing neuromuscular blockade, so large indeed that before this level of sustained depolarization is reached the production of sufficient quantities of AchR after nerve stimulation is interfered with. This then becomes the cause of neuromuscular blockade despite the presence of a slight sustained depolarization. Under these circumstances neostigmine becomes effective as an antagonist, since by preventing the destruction of acetylcholine it will allow a larger concentration of acetylcholine to react with the receptive substance and thus neuromuscular transmission can be restored.

This discussion is much theory based on very

few facts. I hope that this theorizing may open the road for many new experiments.

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Pharmacology of the Anticholinesterase Drugs—Mestinon, Prostigmin, Tensilon and TEPP*

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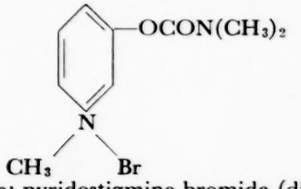
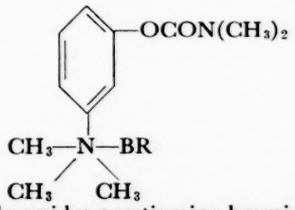
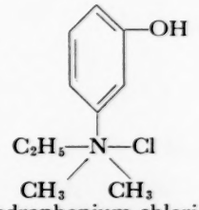
MESTINON® is one of the new anticholinesterase drugs which is being tested currently in myasthenia gravis. A pyridine analog of prostigmin, mestinon was first synthesized by Urban and Schnider (1945)[12] in the Hoffmann-La Roche Laboratories at Basle, Switzerland. It had been found by Osserman et al.[8] and Tether[11] in 1954 to have advantages over prostigmin® in duration of action and mildness of side effects. This paper is concerned with the pharmacology of mestinon in comparison with prostigmin, tensilon® and TEPP (tetraethyl pyrophosphate). Prostigmin has long been the anticholinesterase of choice in the treatment of the disease. Tensilon is a short-acting anticholinesterase which is used as a diagnostic agent (Osserman and Kaplan[7]). TEPP is a representative of the long-acting anticholinesterases which have had unsuccessful trials in the disorder (Grob and Harvey[5]).



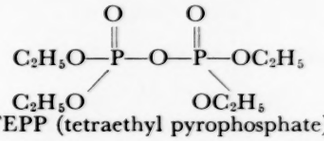
Mestinon bromide; pyridostigmine bromide (dimethyl carbamic ester of 3-hydroxy-1-methylpyridinium bromide)



Prostigmin bromide; neostigmine bromide (dimethyl carbamic ester of 3-hydroxy-phenyl-trimethyl ammonium bromide)



Tensilon chloride; edrophonium chloride (3-hydroxy-phenyl-ethyl-dimethyl ammonium chloride)



TEPP (tetraethyl pyrophosphate)

Toxicity. The acute toxicity of mestinon in comparison with prostigmin was worked out in detail by Fromherz and Pellmont[4] in 1953.

TABLE I
ACUTE TOXICITY IN MICE

	Intravenous		Subcutaneous		Oral	
	LD_{50} (mg./kg.)	Ratio	LD_{50} (mg./kg.)	Ratio	LD_{50} (mg./kg.)	Ratio
Mestinon	1.37	1	2.7	1	32	1
Prostigmin	0.17	8	0.42	6	7.5	4
Tensilon	9.0	1/6	130	1/50	600	1/20
TEPP	0.027	50	.....	...	.....	...

The average toxicity of mestinon was one-fifth that of prostigmin by various routes in mice, rats, rabbits, guinea pigs and cats. The relative toxicities varied from about equal toxicity of mestinon and prostigmin by the subcutaneous route in mice and the oral route in rats to one-fifteenth by the subcutaneous route in rats. The relative toxicities as measured in our laboratory are shown in Table I. Our data are in agreement

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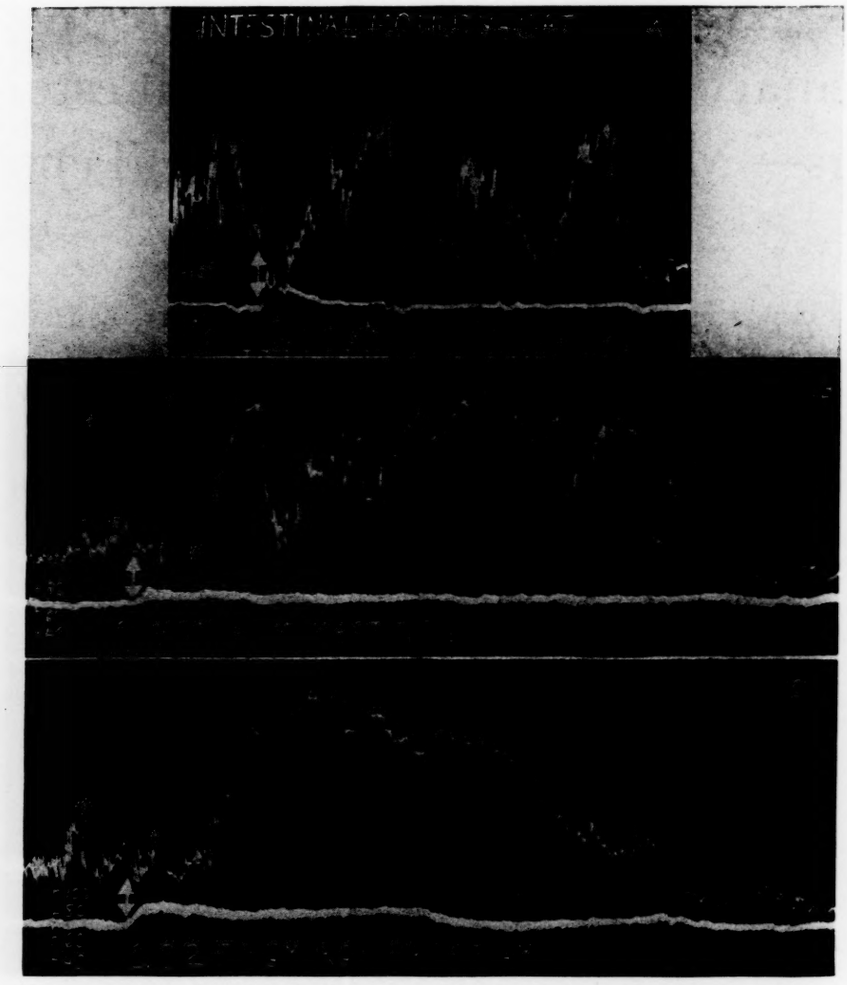

FIG. 1. Comparative stimulating effects of tensilon, mestinon and prostigmin on the intact intestine of the cat. Motility was recorded with a tambour connected by a glass tube to the lumen of the duodenum. Top curve, intestinal motility; middle curve, arterial pressure.

with those of Fromherz and Pellmont (1953) in that mestinon has been found to be one-fourth to one-eighth as toxic as prostigmin. Tensilon is one-sixth to one-fiftieth as toxic as mestinon, while TEPP is fifty times more toxic than mestinon. All the compounds produce typical signs of over-stimulation by acetylcholine—salivation, increased intestinal motility, defecation, fibrillation, convulsions and respiratory paralysis.

Intestinal Motility. Mestinon has about one-fourth to one-half the potency of prostigmin as a stimulant of isolated rabbit intestine (Fromherz and Pellmont[4]). It sensitizes the isolated intestine to acetylcholine and the effects are blocked by atropine. The intact rabbit intestine is also stimulated by mestinon administered intravenously at doses two to four times those of prostigmin and the effects of acetylcholine are potentiated. The intact intestine of the cat also is stimulated by this dosage. In Figure 1 it is shown that 0.1 mg./kg. of tensilon administered intravenously produces a very rapid stimulation followed quickly by relaxation. Mestinon and prostigmin stimulate after a delay of several minutes. Mestinon is about one-fourth as active as prostigmin and the duration of action is at least as long. TEPP has a potency similar to that of prostigmin but the duration of action is longer.

Effect on Blood Pressure. Fromherz and Pellmont[4] in 1953 reported that mestinon and prostigmin have very little effect on blood pressure of cats in small non-toxic doses. Large

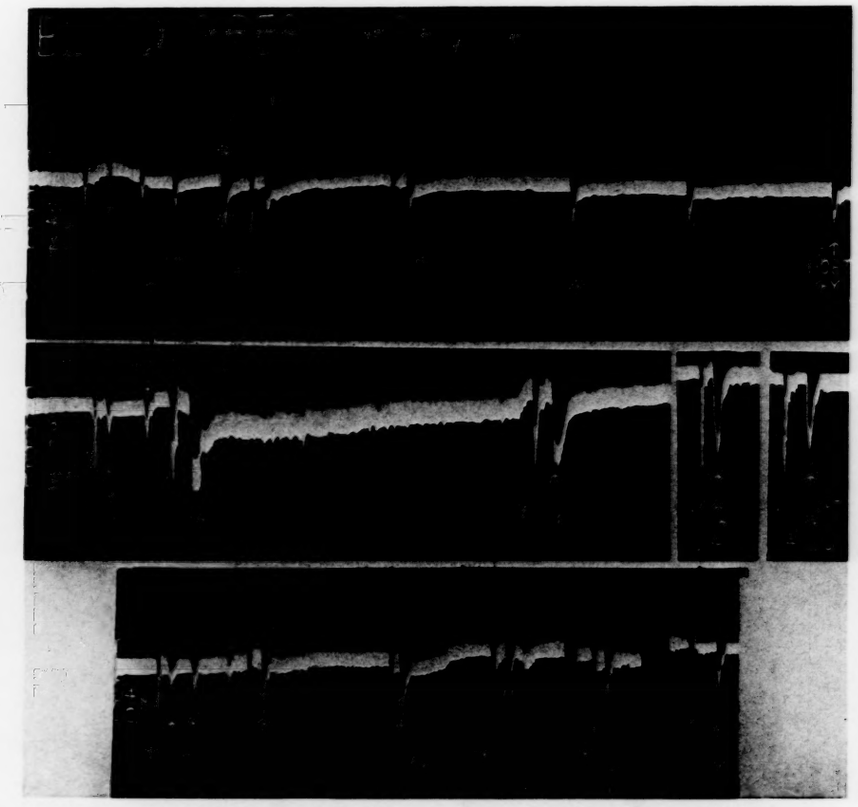

FIG. 2. **Potentiation effects of tensilon, mestinon and prostigmin on the responses of the blood pressure to acetylcholine and vagus stimulation. Blood pressure was measured from the carotid artery of a dog anesthetized with pentobarbital.**

doses cause a fall of blood pressure as a result of parasympathetic stimulation. This is accompanied by dilation of peripheral vessels. Hobbiger[6] in 1950 found that mestinon is one-fifth as active as prostigmin as a sensitizer of the frog rectus to acetylcholine while TEPP is equal to prostigmin.

Our experience confirms the slight effects of mestinon on the blood pressure of cats. (Fig. 1.) The results of potentiation experiments on acetylcholine and the vagus in the dog are shown in Figure 2. Tensilon potentiates the depressor effects of acetylcholine for about fifteen minutes but the depressor response to vagus stimulation is not potentiated. Mestinon and prostigmin potentiate both effects for several hours. Mestinon is not more than one-fourth as active as prostigmin. There is a prolonged potentiation of these effects by TEPP. This compound is a stronger cholinergic agent than prostigmin and is also much more toxic.

Neuromuscular Transmission. Fromherz and Pellmont[4] in 1953 found that mestinon has about one-half of the anticurare activity of prostigmin in cats. The paralysis of the sciatic tibial preparation produced by tubocurarine was antagonized by 0.2 mg./kg. of mestinon or 0.1 mg./kg. of prostigmin. Casier and Verbeke[3] in 1950 also observed anticurare action in the same dose range. Blaschko et al.[1] in 1949 used the rat phrenic diaphragm preparation and found that mestinon was $\frac{1}{40}$ as strong as prostigmin. Hobbiger[6] in 1950 reported results indicating that the effect of mestinon as an anticurare agent was one-fifth as strong as prostigmin on the frog rectus. TEPP was equal to prostigmin.

Our experiments (Fig. 3) show that mestinon has about one-fourth the activity of prostigmin as an antagonist to tubocurarine paralysis of the sciatic nerve tibial muscle preparation in cats. Tensilon is as active as mestinon but TEPP is weaker. Tensilon has a rapid onset and a short duration of action whereas mestinon, prostigmin and TEPP have long duration and slow onset.

The duration of action in these experiments was estimated from the time required for repeated doses of tubocurarine to reproduce the original block. While tensilon effects were

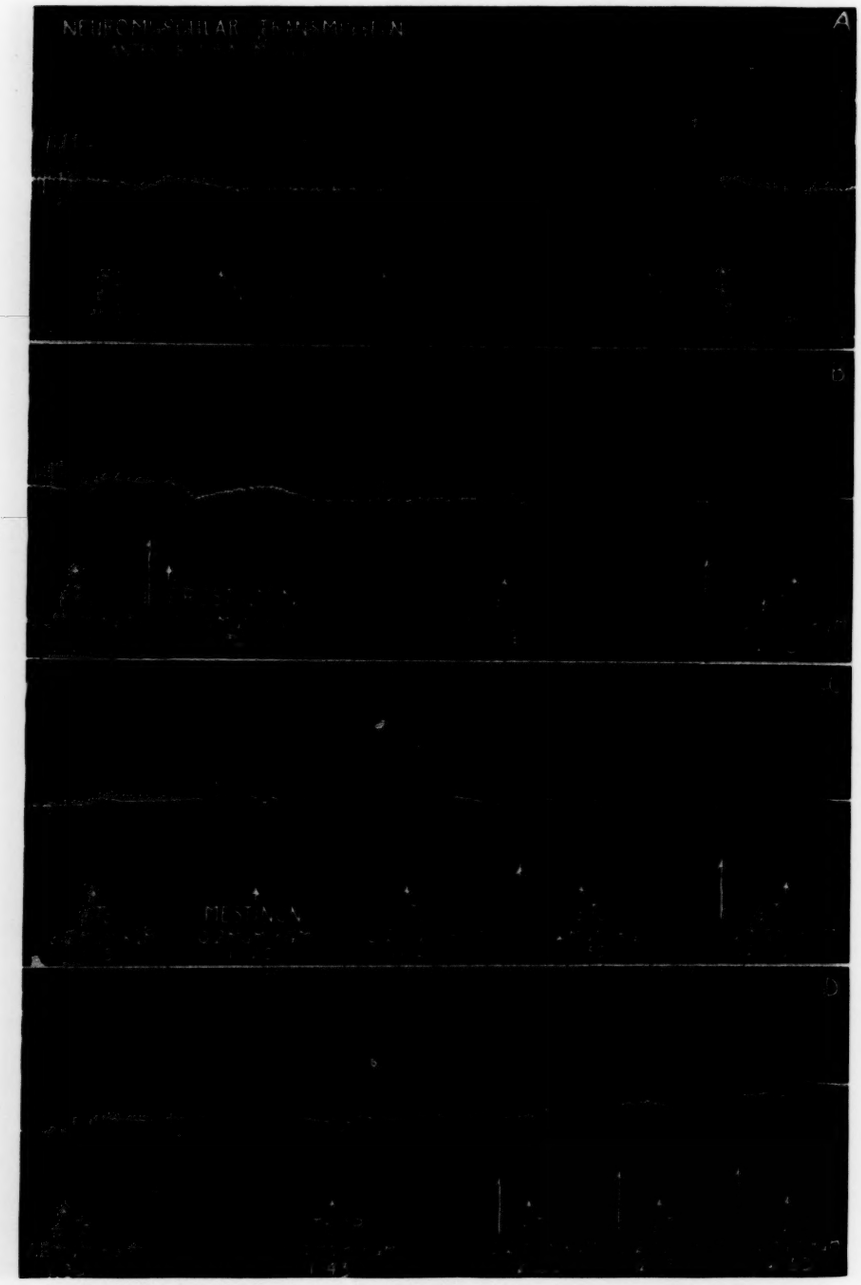

FIG. 3. Anticurare action of tensilon, prostigmin, mestinon and TEPP in a cat anesthetized with pentobarbital. Recording of the anterior tibial muscle stimulated through the sciatic nerve; time is in minutes. At the long arrows, part of the record was omitted.

counteracted by *d*-tubocurarine within ten minutes, the action of mestinon, prostigmin and TEPP lasted up to an hour.

Potentiation of the twitch tension by tensilon is also a rapid action of short duration. (Fig. 4.) Mestinon and prostigmin have a greater delay of onset and a gradual effect on twitch tension. Mestinon is about one-eighth as strong as prostigmin in this test and the duration of effects are about equal. TEPP is relatively weak and it has a much delayed onset of action. The spread between the doses which produces an increase in tension and the curariform effect also differ. This spread between the potentiating dose and the curarizing dose represents the safety margin of the drugs. Tensilon has an eight-fold safety

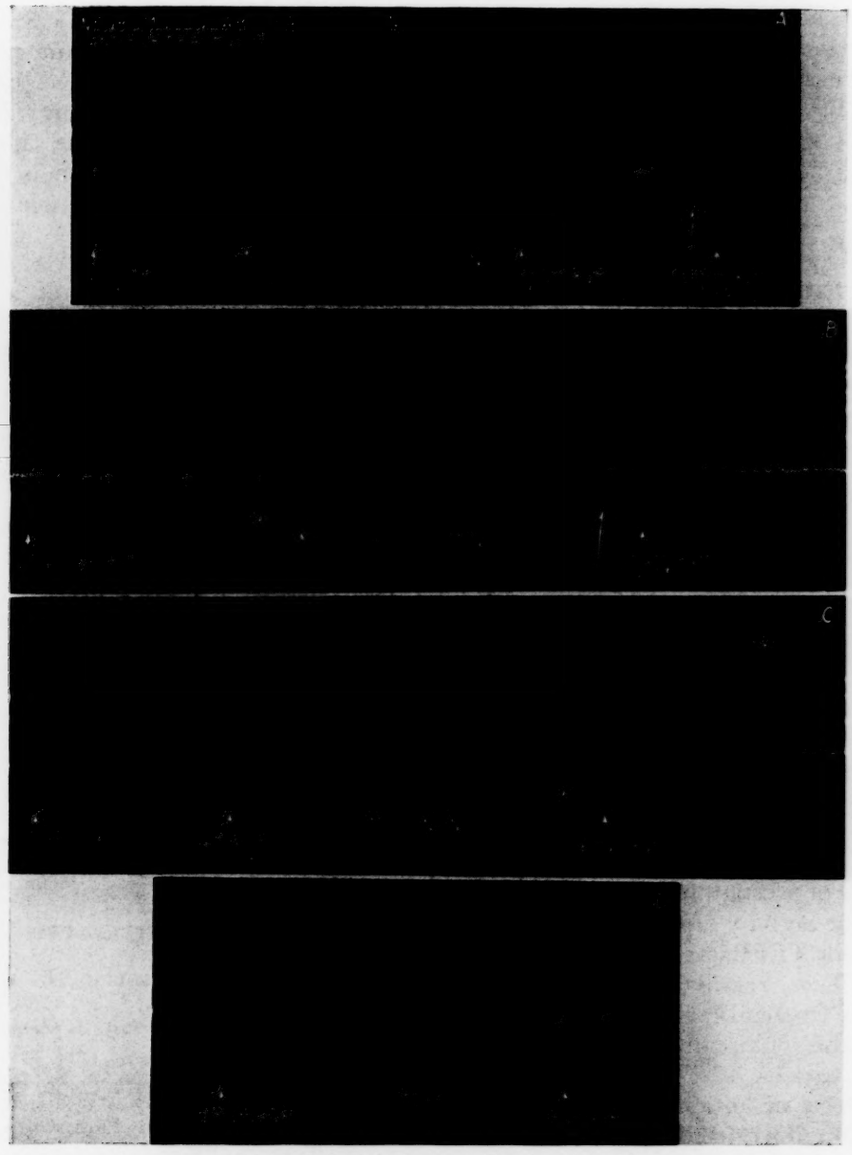

FIG. 4. Potentiation of the twitch tension in cats by tensilon, prostigmin, mestinon and TEPP. Recording of the response of the tibial muscle to stimulation of the sciatic nerve.

margin, prostigmin a four-fold safety margin, mestinon at least an eight-fold safety margin, while TEPP has only a two-fold safety margin. Mestinon has a greater safety margin than prostigmin in this test.

The differences in rate of onset of action in these experiments may represent the differences in the rate of penetration or diffusion to the site of action at the neuromuscular junction. The increase in tension produced by the agents as well as the curariform effect are interpreted as the result of the accumulation of acetylcholine at the sites of action. Alternate explanations for the fast action of tensilon are that it may have a direct depolarizing action at the neuromuscular junction and it may potentiate acetylcholine directly. However, most workers now believe that the action is primarily an anticholinesterase effect with resultant accumulation of acetylcholine. This subject has been reviewed by Riker[10] (1953) and Randall and Jampolsky[9] (1953).

Anticholinesterase Activity. The anticholinesterase activity of mestinon was found to be one-tenth that of prostigmin by Blaschko et al.[1] in 1949. This relative activity was measured *in*

vitro using true cholinesterase from the brain of the dog. The relative anticurare activity on the phrenic diaphragm of the rat was one-fortieth. Hobbiger[6] in 1950 indicated that mestinon had about one-fifth the activity of prostigmin in inhibiting the cholinesterase of frog muscle.

TABLE II
CORRELATION OF CHOLINESTERASE INHIBITION WITH TOXICITY AND ACTIVITY

	Cholinesterase Inhibition		Relative Toxicity in Mice (intravenously)	Relative Anticurare Activity
	Concentration for 50% Inhibition	Ratio		
Prostigmin	1×10^{-7}	1	1	1
Mestinon	2×10^{-6}	1/20	1/8	1/4
Tensilon	2×10^{-5}	1/200	1/50	1/4
TEPP	5×10^{-9}	20	6	1/8

This same ratio of activity was found for the sensitizing of muscle to acetylcholine and for the anticurare effect on frog muscle. Casier[2] in 1950 also reported on low anticholinesterase activity of mestinon with respect to true cholinesterase.

Our results indicate that mestinon has about 1/20 the activity of prostigmin when tested on red cell esterase; the activity of tensilon was 1/200 of prostigmin, while TEPP was twenty times that of prostigmin. These relative anticholinesterase values correlate reasonably well with the relative toxicities of the compounds in mice. This indicates that toxicity is a consequence of the cholinergic effects of anticholinesterase agents. The relative anticurare activities do not correlate with the anticholinesterase values or the toxicity. Mestinon itself does show a slightly better ratio of anticurare activity to toxicity and anticholinesterase activity than prostigmin. Tensilon shows a high anticurare activity in relation to anticholinesterase activity and toxicity, while TEPP has a very poor ratio of anticurare activity to toxicity and anticholinesterase activity. The anticurare activities are better correlated with the clinical results than either toxicity or anticholinesterase activities. (Table II.)

These anticurare results correlated with the clinical findings in myasthenia gravis reveal that mestinon is better tolerated than prostigmin although it is a weaker drug. Tensilon is very well tolerated and it is quite safe in myasthenia gravis; its very short action is useful for diagnostic purposes. TEPP is useless because of its low margin of safety.

SUMMARY

Mestinon is a pyridine analog of prostigmin which has one-eighth to one-fourth the toxicity of prostigmin, one-fourth the intestinal stimulating capacity, one-fourth the potentiating effects on acetylcholine and the vagus, one-fourth the anticurare activity, one-eighth the potentiating action on twitch tension and one-twentieth the anticholinesterase activity on red cell esterase. Mestinon and prostigmin have a slow onset of action in various tests and a long duration of action in contrast to the rapid onset and short duration of action of tensilon. Mestinon and prostigmin have a much better safety margin between anticurare activity and toxicity than TEPP. The anticurare activity in cats offers a better index of predictability of clinical activity in myasthenia gravis than the toxicity or anticholinesterase activity of these compounds.

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Electromyographic Changes in Myasthenia Gravis*

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A COMPLETE description of the partial block in neuromuscular transmission in myasthenia gravis is a prerequisite in any attempt to uncover the underlying cause of this block. The purpose of this study is to describe in some detail the properties of the myasthenic neuromuscular block. This was accomplished by recording the electrical response of muscle to various patterns of nerve stimulation.

The ulnar nerve was stimulated percutaneously just above the elbow with a rectangular current pulse of 150 microseconds' duration. Great care was taken to insure maximal nerve stimulation at all times. The electrical activity of the muscle was recorded between a surface electrode over the belly of the abductor of the fifth finger and an indifferent electrode. These muscle action potentials were amplified and photographed from the face of a cathode-ray oscillograph. Such action potentials are polyphasic and represent a summation of the individual muscle fiber action potentials in the region of the recording electrode. (Fig. 1.) Thus when some neuromuscular junctions are blocked their individual muscle fibers do not respond to the nerve stimulus and the summated or integrated action potential decreases in size. Contrariwise, when there is a decrease in neuromuscular block more individual muscle fibers respond, and the integrated action potential increases in size. Changes in the size of the integrated action potential therefore serve as a measure of changes in neuromuscular transmission.

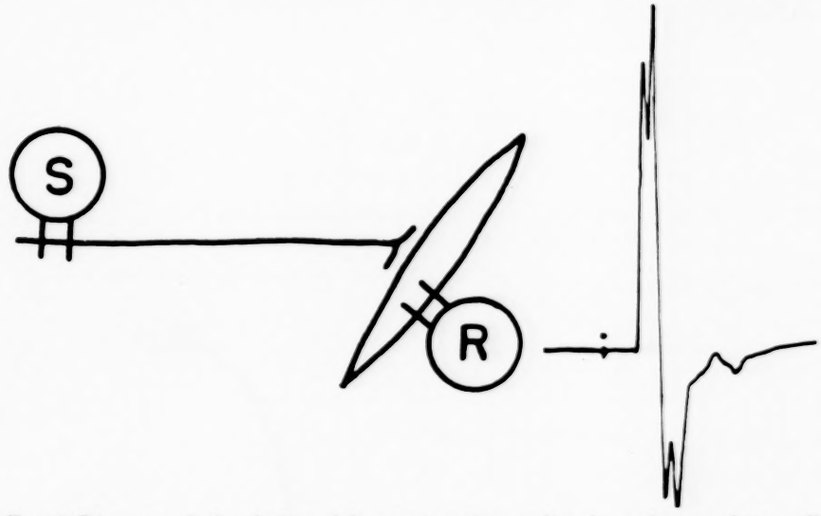

FIG. 1. Diagram of stimulation of the nerve and recording from the muscle as well as a photograph of a typical muscle action potential.

Is there a partial block of the transmission of a single impulse in generalized myasthenia gravis? This question was investigated by comparing the size of the action potential in response to a single nerve stimulus in a group of normal subjects and a group of myasthenic patients. The mean amplitude in a group of twenty normal subjects was 7.5 millivolts; in a group of twenty-six patients with generalized myasthenia gravis it was 5.4 millivolts. While the difference be-

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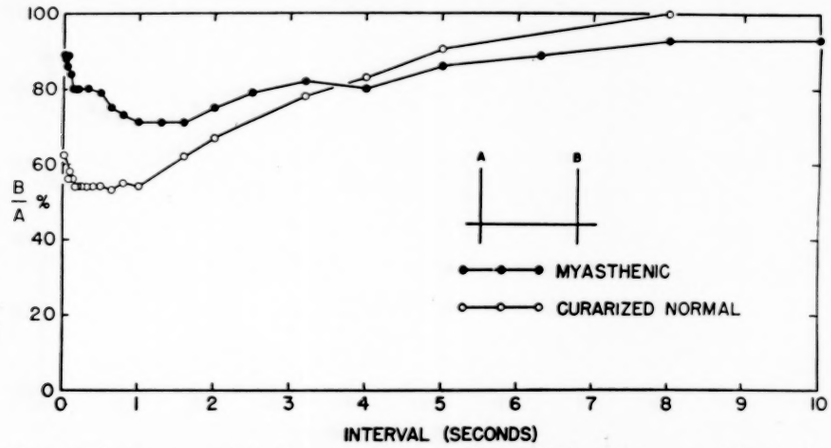

FIG. 2. Time course of the increased degree of block following passage of a single impulse in generalized myasthenia gravis (closed circles) and partial curarization (open circles). The size of the test response (B) is expressed as a percentage of the conditioning response (A) and is plotted against the interval between the responses.

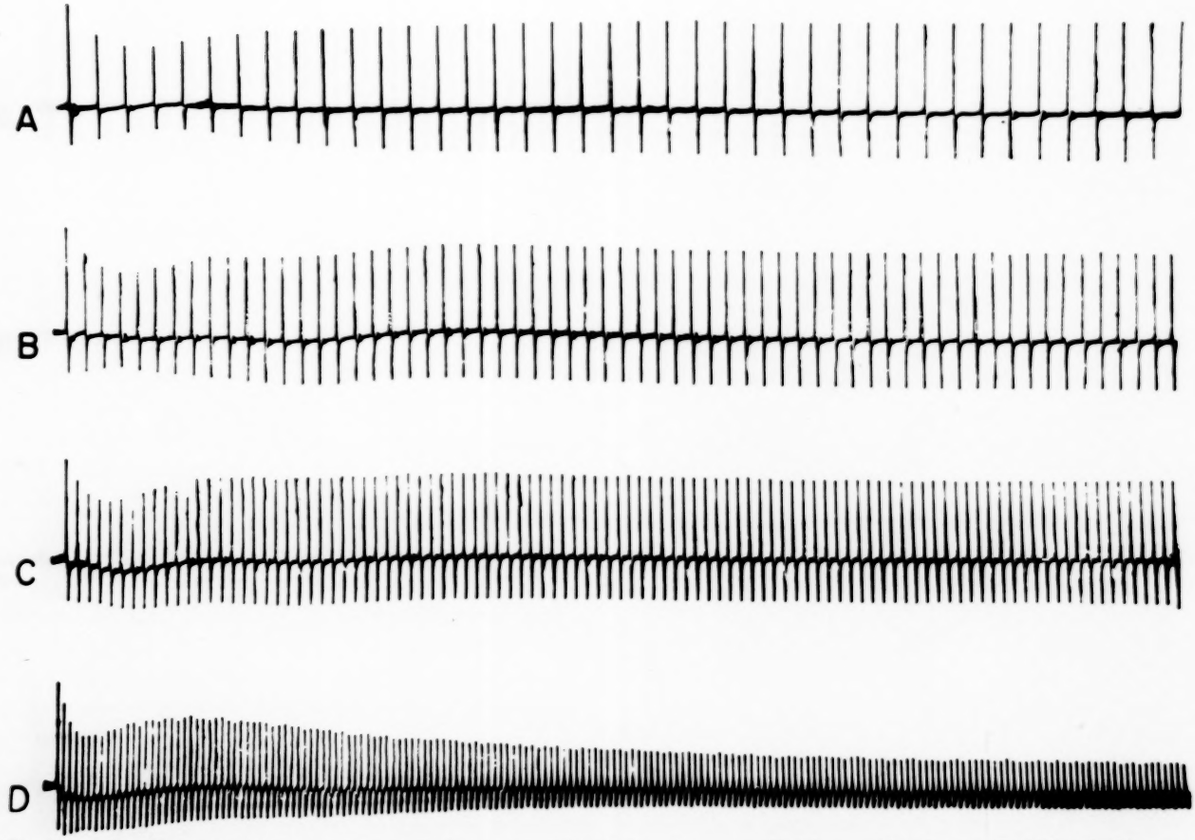

FIG. 3. Action potential response to repetitive nerve stimulation in generalized myasthenia gravis. Stimulation at rates of A, 5 per second; B, 10 per second; C, 25 per second, and D, 50 per second. The late exponential increase in degree of block has not yet occurred in the segments of the record shown in A and B.

tween these means was statistically significant, there was a wide overlap between the individual values in the two groups. Thus statistical analysis suggests that there is a partial block of the transmission of a single impulse in the myasthenic group but it provides no information regarding the presence or absence of block in many of the individual myasthenic patients whose potentials were within the broad normal range. The fact that neostigmine can increase the size of the action potential response to a single stimulus in most patients with generalized myasthenia gravis, even when the potential is within the normal range, may be taken to indicate that some degree of block was present in these patients.

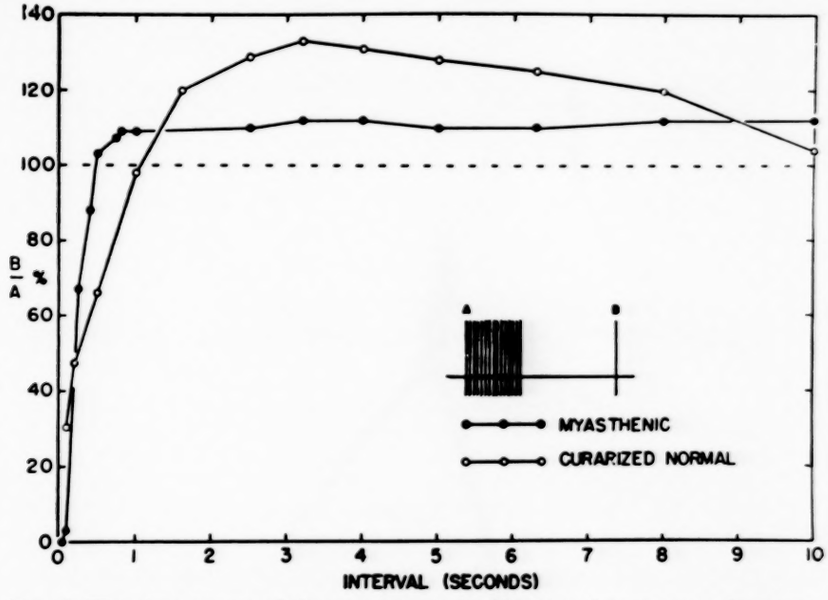

FIG. 4. The time course of the post-tetanic facilitation of neuromuscular transmission in generalized myasthenia gravis (closed circles). The conditioning tetanus was a train of 121 stimuli at 200 per second. The size of the test response (B) is expressed as a percentage of the size of a pretetanic response (A). This is plotted against the interval between the end of the conditioning train and the test stimulus. Similar results are obtained in a partially curarized normal subject (open circles).

The effect of transmission of a single impulse on the ability of the myasthenic neuromuscular junctions to transmit a subsequent impulse was next investigated. A conditioning nerve stimulus was followed by a test stimulus and the test action potential response was compared to the conditioning response. In patients with generalized myasthenia gravis the second (test) response was smaller than the first (conditioning) response, demonstrating that there was an increased degree of block to the passage of the second response. The time course of this increased block following the passage of a single impulse was traced out by varying the interval between conditioning and test stimuli. (Fig. 2.) The block increased following the passage of an impulse and reached a maximum in about one second. The degree of block then gradually decreased. Transmission did not return to the control level until ten or more seconds after the conditioning nerve stimulus. No such block was demonstrated in normal subjects but was reproduced in the partially curarized normal subject.

When trains of stimuli were delivered to the ulnar nerve at various frequencies, there was a characteristic time course of increase in degree of block, as evidenced by the size of the action potential response to the successive stimuli. (Fig. 3.) At first there was an abrupt increase in block followed by a period of decreased block. This was in turn followed by a slow exponential increase in block. A similar sequence of events was demonstrated in partially curarized normal subjects. In contrast, in normal subjects the amplitude of the action potentials was maintained in response to stimulation at rates up to 25 per second.

Neuromuscular transmission during the period

following a train of repetitive stimuli was studied in the same manner described for the period following a single stimulus. A conditioning train was delivered. At variable time intervals after the end of the train a single test stimulus was applied. The muscle action potential in response to the single test stimulus was compared with the response to a single stimulus delivered before the tetanic stimulation. The response to the first stimulus of the conditioning train of stimuli was used for this pre-tetanic control. Again the time course of this post-tetanic change in degree of block was obtained by varying the interval between the end of the tetanus and the test response. (Fig. 4.) After one second there was a period of facilitation of neuromuscular transmission during which the test response exceeded the control response. This post-tetanic facilitation (or decrease in degree of block) disappeared after approximately 10 seconds. Increases in either duration or frequency of the conditioning train increased the degree of facilitation. With a conditioning train frequency of 100 per second there may be discernible action potential responses to only the first 20 to 30 stimuli due to the rapid and marked increase in degree of block at such high frequencies. Nevertheless further increases in train length or frequency were capable of increasing the degree of post-tetanic facilitation. This fact indicates that postjunctional events, initiation and propagation of the muscle action potential and muscular contraction, were not essential to the production of post-tetanic facilitation. Post-tetanic facilitation of neuromuscular transmission could not be demonstrated in normal subjects, nor would facilitation be expected since neuromuscular transmission is already maximal. However, post-tetanic facilitation (or decurarization) could be seen in the partially curarized normal subject. (Fig. 4.)

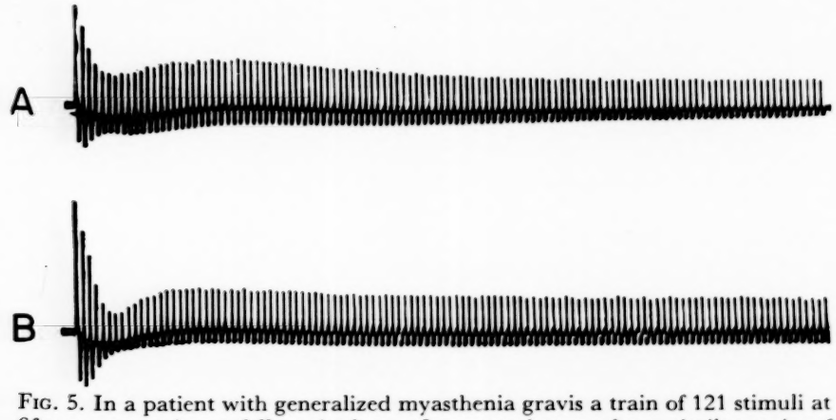

FIG. 5. In a patient with generalized myasthenia gravis a train of 121 stimuli at fifty per second was followed after a five second pause by a similar train of stimuli. A, the response to the first train showing the typical decreased muscle action potential size; B, the response to the second train showing post-tetanic facilitation of the first action potential followed by more rapid decline of subsequent potentials.

In order to investigate this myasthenic post-tetanic facilitation further the conditioning train of stimuli was followed at different time intervals by a test train of stimuli. Again the first action potential response to the test train exhibited post-tetanic facilitation but subsequent responses declined more rapidly. (Fig. 5.) Thus the post-tetanic facilitation of transmission of a single impulse appeared to be at the expense of the ability to transmit subsequent impulses.

SUMMARY

The partial block of transmission at the neuromuscular junction in generalized myasthenia gravis manifests several characteristic features.

1. There is a slight degree of block to the passage of a single impulse.

2. Following the passage of a single impulse there is an increase in the degree of block which reaches a maximum about one second after the passage of the impulse. Ten seconds may be required before the degree of block decreases to its initial level.

3. The application of a train of impulses to the neuromuscular junction results first in a progressive increase in block to the passage of the impulses. This is followed by a transient decrease in block which in turn is followed by a progressive increase in degree of block. The

magnitude of this increase in block increases as the stimulating frequency is raised.

4. Repetitive nerve stimulation is followed after a brief interval by a period of facilitation of neuromuscular transmission. This post-tetanic facilitation represents a decrease in the myasthenic block and is probably prejunctional in origin. The degree of post-tetanic facilitation is increased by increasing the duration or frequency of the tetanic stimulation. The post-tetanic facilitation of transmission of a single impulse is at the expense of the myasthenic subject's ability to transmit subsequent impulses.

While such a study provides no direct evidence regarding the cause of the myasthenic block, it does afford a detailed description of this block. Such a description provides a basis upon which comparisons can be made between the myasthenic block and pharmacologically produced blocks; it gives a frame of reference against which drug-produced changes in the myasthenic block may be measured. The close resemblance between the neuromuscular block in myasthenia gravis and the block produced by *d*-tubocurarine in normal subjects has been pointed out. This indicates that the myasthenic block could be produced by a competitive (or curare-like) block.

Alterations in Neuromuscular Transmission in Myasthenia Gravis As Determined by Studies of Drug Action*

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It is now generally accepted that neuromuscular transmission is normally mediated by acetylcholine (ACh), which is released from the motor nerve ending following stimulation of the nerve. ACh depolarizes the muscle endplate, initiating the endplate potential, which in turn initiates the propagated action potential and contraction of the muscle fiber. The ACh is normally quickly hydrolyzed into acetate and choline by cholinesterase concentrated in the region of the endplate, following which repolarization occurs and the endplate is thereby made ready to be acted upon again.

Impairment of transmission at the neuromuscular junction, i.e., neuromuscular block, may result from either excessive or deficient action of ACh on the endplate, or from drugs which simulate these effects. The administration of ACh in excessive concentration, or of an anticholinesterase compound such as neostigmine which permits the local accumulation of an excessive concentration of endogenous ACh, results in abnormally prolonged depolarization of the endplate. While it is depolarized the endplate cannot be stimulated, and neuromuscular block ensues until the ACh is hydrolyzed. This "depolarization" type of block, which also occurs in most animal species following the administration of decamethonium or choline, is intensified by the administration of ACh or neostigmine. A second type of block is the "competitive" block. This results following the administration of *d*-tubocurarine which blocks the access to the endplate of ACh released from the motor nerve ending, probably by competing with ACh for a common receptor site in the endplate. The competitive type of block inhibits the depolarizing action of ACh more completely than does the depolarizing type of block. The competitive type of block is reversed following the administration of ACh or neostigmine, which permits the local accumulation of sufficient ACh to displace *d*-tubocurarine and overcome the block.

In an effort to obtain further information on the characteristics of neuromuscular transmission in normal subjects and in patients with myasthenia gravis, a study was made of the effect of ACh on neuromuscular transmission. This was done by injecting ACh into the brachial artery through an indwelling cannula during intermittent supramaximal stimulation of the ulnar nerve and observing the effect on the amplitude of the induced muscle action potentials recorded from the skin over the abductor of the fifth finger. Trains of four supramaximal stimuli were delivered to the ulnar nerve at intervals of 40 milliseconds every two to five seconds and injections made after the fourth train. Stimulation was continued at two- to five-second intervals for several minutes, and then at longer intervals for periods up to several hours. Observations were carried out in twenty-five normal subjects and fifteen patients with generalized myasthenia gravis.

In each of the normal subjects ACh, in doses of 1 mg. or more, produced a brief burst of potentials, accompanied by involuntary movement, lasting five to ten seconds. Immediately afterward there occurred a period of "prompt" depression of induced potentials, lasting about twenty seconds, with even depression of successive potentials. (Figs. 1 and 2.) This was followed by temporary recovery and then by a more prolonged "late" depression of induced potentials which began one to three minutes

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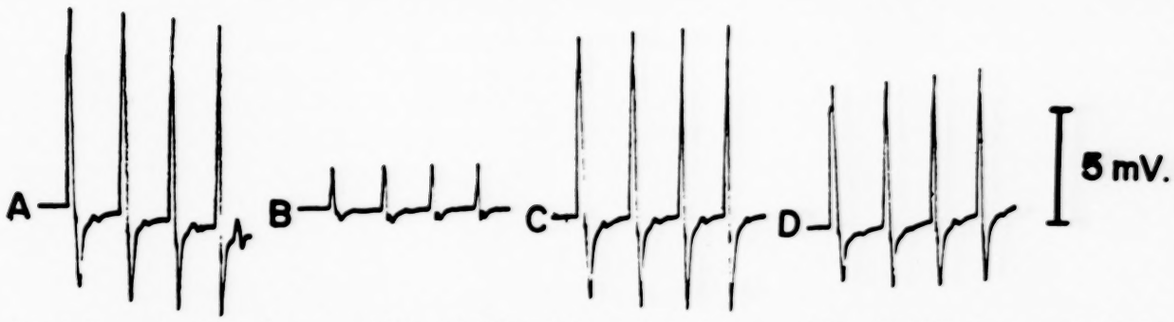


FIG. 1. Prompt and late depressant effects of ACh on the muscle action potentials evoked by nerve stimulation in a normal subject. A, control muscle potentials in response to four supramaximal nerve stimuli at forty-millisecond intervals. B, prompt depression eight seconds after the intra-arterial injection of 5 mg. ACh. C, recovery fifteen seconds after injection. D, late depression twenty-three minutes after injection.

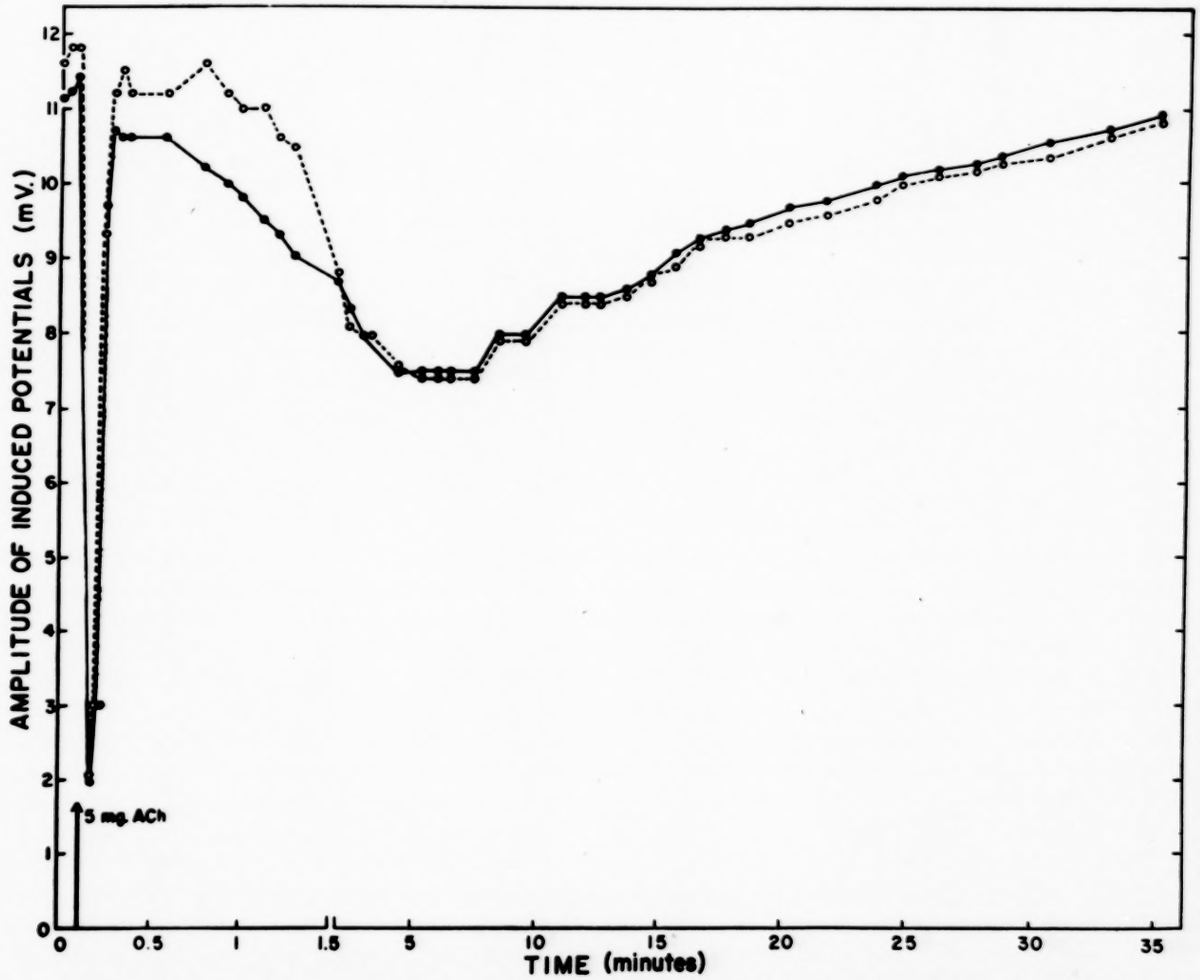


FIG. 2. Time course of the prompt and late depressant effects of intra-arterially injected ACh on the muscle action potential responses to nerve stimulation in a normal subject. The nerve was stimulated by trains of four supramaximal stimuli forty milliseconds apart at two-second intervals. The muscle action potentials in response to the first (●—●) and fourth (○----○) nerve stimuli of each train have been plotted.

after the injection of ACh and lasted for one-half to one hour. Successive potentials were evenly depressed.

The properties of the prompt and late depressions (i.e., neuromuscular blocks) produced by ACh were investigated by observing the effect of sodium acetate, in doses up to 200 mg., had no effect on induced potentials. The intra-arterial injection of choline chloride in doses of 5 to 30 mg. produced prolonged depression of induced potentials which resembled the late depression produced by ACh. Successive potentials were evenly depressed. The depression produced by these doses of choline was enhanced by the injection of ACh or neostigmine (Fig. 5), indicating that it too is a depolarizing type of block. Larger doses of choline produced more marked depression than occurred (late) following ACh. This was enhanced by the injection of ACh, but reversed to a slight extent by small doses of neostigmine, manifesting properties of a mixed type of block. The depression produced by choline differed from the late depressant effect of ACh in its more rapid onset (ten seconds after injection), but otherwise the time course was the same. It seems likely that the late depression produced by ACh is due to choline liberated following the hydrolysis of ACh, and that the longer latent period represents the time required for hydrolysis to occur. The main difference between the depressant effect of choline and of

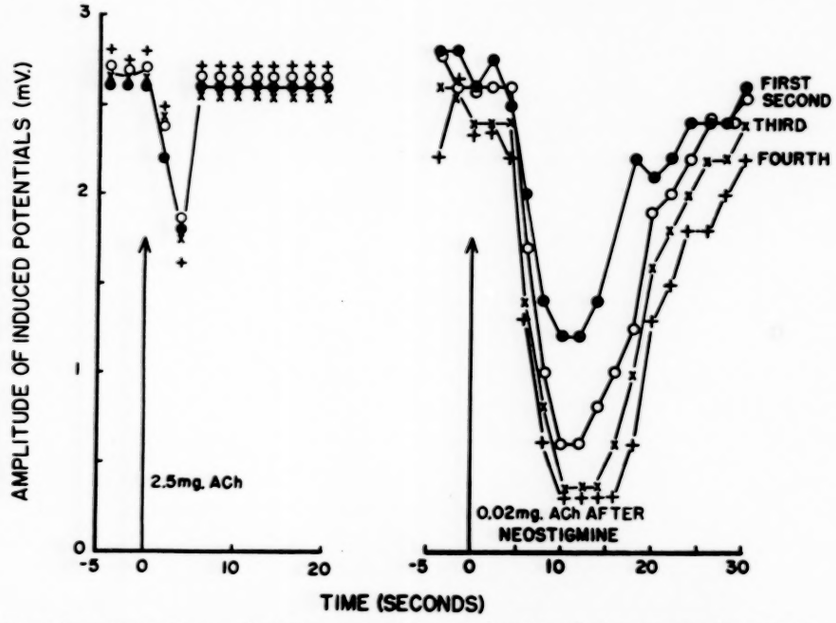
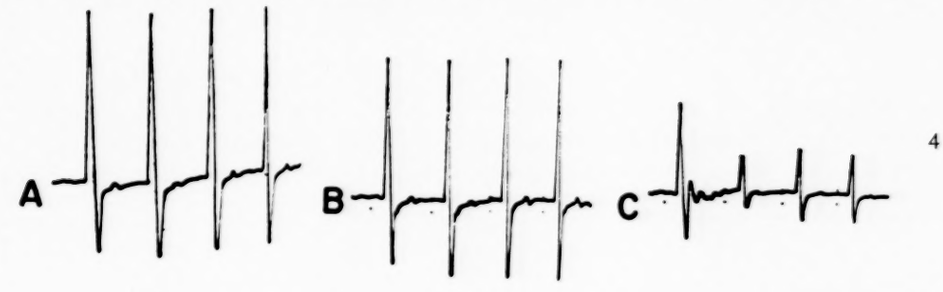


FIG. 3. Potentiation and prolongation by neostigmine of the prompt depressant action of intra-arterial ACh on muscle action potentials induced by nerve stimulation in a normal subject. The nerve was stimulated by trains of four supramaximal stimuli forty milliseconds apart at two-second intervals. On the left is shown the prompt depressant effect of 2.5 mg. ACh prior to neostigmine, and on the right of 0.02 mg. ACh administered twelve minutes after the intra-arterial injection of 1.2 mg. neostigmine. Prior to neostigmine, 0.02 mg. ACh had no effect on the induced potentials.

the intra-arterial injection of ACh and neostigmine on each block. The prompt depression was enhanced and prolonged by the prior injection of neostigmine (Fig. 3), indicating that it has the properties of a depolarizing block. The late depression was also increased following the administration of ACh or neostigmine (Fig. 4), indicating that this too has the properties of a depolarizing type of block. The time course of the prompt depression produced by ACh is compatible with this being a direct effect of ACh on the motor endplates, since this compound is rapidly hydrolyzed. Since the late depression did not begin until one to three minutes after the injection of ACh and since it persisted for one-half to one hour, long after hydrolysis of the ACh had occurred, the possibility that it might be due to one of the products of hydrolysis of ACh was investigated. The intra-arterial injection of



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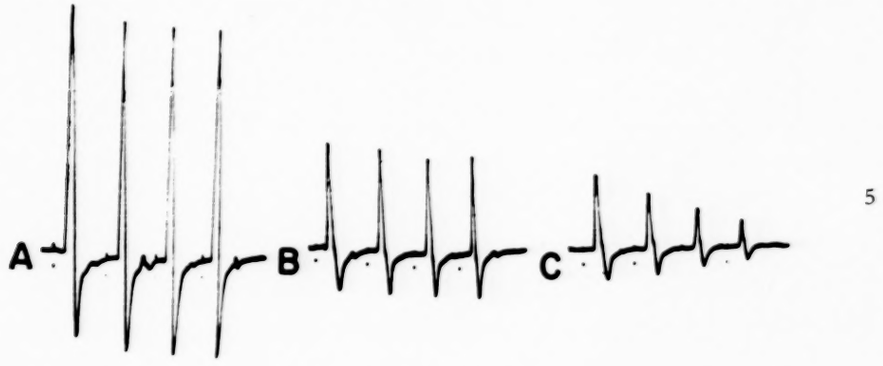

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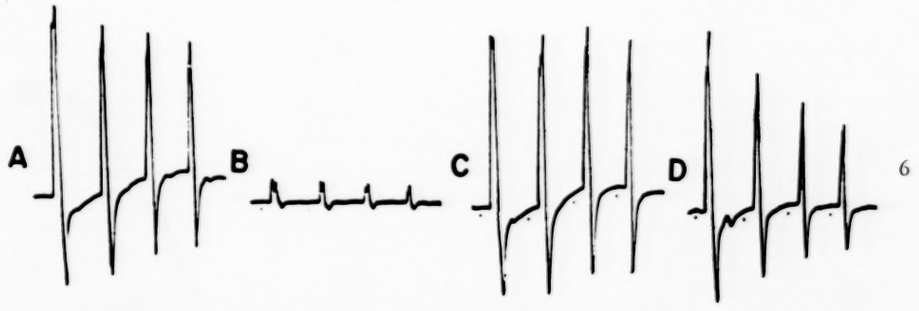

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FIG. 4. Depressant effect on induced muscle action potentials of neostigmine administered following their (late) depression by ACh in a normal subject. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, late depression following the intra-arterial injection of four doses of 10 mg. ACh. C, further progressive depression and repetitive firing after the initial potential, thirty-five seconds after the intra-arterial injection of 0.5 mg. neostigmine.

FIG. 5. Depressant effect on induced muscle action potentials of neostigmine administered following their depression by choline in a normal subject. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, depression produced by the intra-arterial injection of 30 mg. choline. C, further progressive depression seventy-five seconds after the intra-arterial injection of 1 mg. neostigmine.

FIG. 6. Prompt and late depressant and reparative effects of ACh on the muscle action potential responses to nerve stimulation in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, prompt depression ten seconds after the intra-arterial injection of 5 mg. ACh. C, repair of the myasthenic defect twenty-two seconds after injection. D, late depression seven minutes after injection.

ACh was in the dose-effect relationship, similar degrees of depression being produced by choline in doses three times that of ACh. Since each milligram of ACh yields 0.8 mg. of choline on hydrolysis, one might expect the reverse to occur. However, it is also possible that ACh these potentials was more marked. As in the normal subjects the prompt depression was enhanced by the prior injection of neostigmine, indicating that it is a depolarizing type of block. On the other hand, the late depression produced by ACh was reversed by the administration of

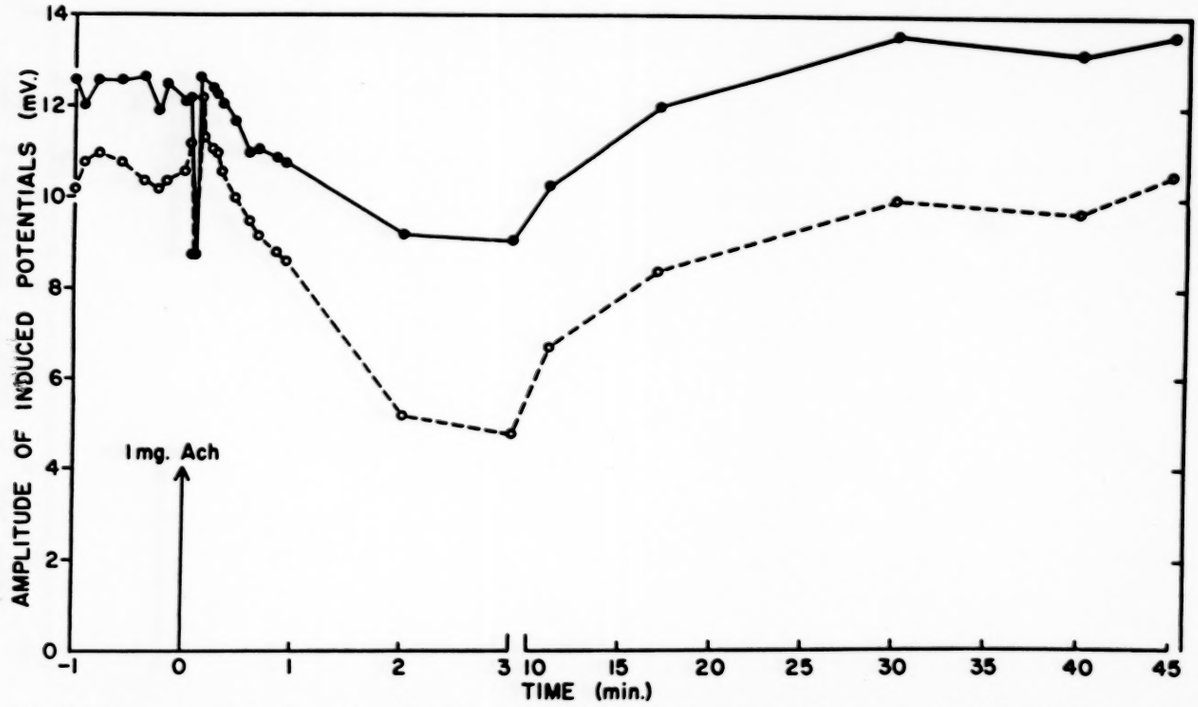
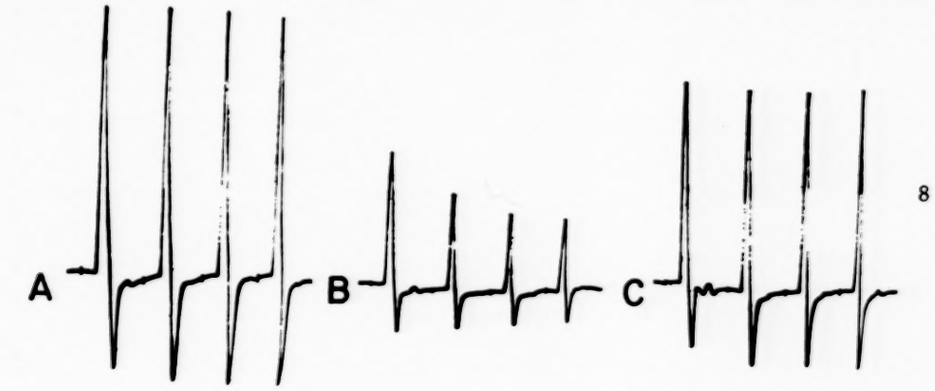


FIG. 7. Time course of the prompt and late depressant and reparative effects of intra-arterially injected ACh on the muscle action potential responses to nerve stimulation in a myasthenic patient. The nerve was stimulated by trains of four supramaximal stimuli at five-second intervals. The muscle action potentials in response to the first (●—●) and fourth (○----○) nerve stimuli of each train have been plotted.

hydrolyzed at the neuromuscular junction may give rise to choline in more intimate contact with the endplates than would result from the injection of choline.

In each of the patients with myasthenia gravis the injection of ACh, in doses of 1 mg. or more, produced transient stimulation of motor unit activity and involuntary movement, followed by prompt and late depressions of induced potentials having the same time course as in normal subjects. (Figs. 6 and 7.) In addition there was transient increase in amplitude, or repair, of the latter potentials which had been initially reduced in amplitude, manifesting the decrement characteristic of myasthenia gravis. This repair occurred between the prompt and late depressions, and lasted for two minutes. The degree of prompt depression of the latter potentials of a train was less in myasthenic patients than in normal subjects, while the late depression of ACh or neostigmine (Fig. 8), indicating that it is a competitive type of block in the myasthenic patients, in contrast to the depolarizing type of block which occurred in normal subjects. The time course of the late depression produced by ACh again suggested that this depression might be due to one of the products of hydrolysis of ACh. Once more sodium acetate had no effect but the intra-arterial injection of choline chloride, in doses of 5 to 30 mg., produced transient increase in the induced potentials followed by prolonged depression which resembled the late depressant effect of ACh. The depression produced by choline, which was more marked in the later potentials of a train in myasthenic than in normal subjects, was promptly reversed by ACh or neostigmine (Fig. 9), indicating that it is a competitive type of block in myasthenic patients. The late depression produced by ACh would therefore appear to be due to choline

released following the hydrolysis of ACh. As in the normal subjects, approximately three times as much choline as ACh was required to produce comparable degrees of depression, again suggesting that choline derived from ACh hydrolyzed at the neuromuscular junction may have more ready access to the endplate than injected choline.



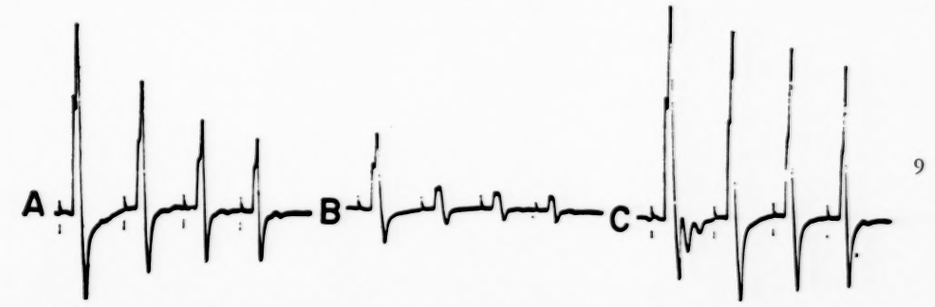


FIG. 8. Reversal by neostigmine of the late depression of induced muscle action potentials produced by ACh in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, late depression following the intra-arterial injection of five doses of 7.5 mg. ACh. C, reversal of the depression two minutes after the intra-arterial injection of 1 mg. neostigmine.

FIG. 9. Reversal by neostigmine of the depression of induced muscle action potentials produced by choline in a myasthenic patient. A, control muscle potentials evoked by four supramaximal nerve stimuli at forty-millisecond intervals. B, depression following the intra-arterial injection of 100 mg. choline. C, reversal of the depression seven minutes after the intra-arterial injection of 1 mg. neostigmine.

It is not known if the observed effects of injected ACh and choline have faithfully reproduced the actions of the endogenous compounds released during neuromuscular transmission. Nevertheless these effects do indicate that the defect in neuromuscular transmission in myasthenia may be due to a competitive block produced by choline released in a normal manner during neuromuscular transmission following hydrolysis of endogenous ACh. The competitive type of block inhibits the depolarizing action of ACh more completely than the depolarizing type of block, and inhibition of the depolarizing action of endogenous ACh released following nerve stimulation would explain the neuromuscular block of myasthenia gravis. It is not yet known why choline produces a competitive type of block in myasthenic patients and a predominantly depolarizing block in normal subjects. Studies are in progress to determine whether this is due to an abnormal response of the myasthenic endplate to choline itself, or to the formation in myasthenic patients of a product of choline which is a competitive blocking agent. Churchill-Davidson and Richardson have found a similar abnormal response of myasthenic patients to decamethonium, and we have likewise observed that decamethonium produces a competitive type of block in these patients, in contrast to a predominantly depolarizing block

in normal subjects. This suggests that the myasthenic endplate may react abnormally to a variety of compounds which normally produce depolarization, the response to choline being of particular importance because of the release of this compound following motor nerve activity, ACh release and hydrolysis.

SUMMARY

The injection of ACh was found to produce, in normal subjects and in patients with myasthenia gravis, transient stimulation of motor unit activity followed by prompt transient depression of induced potentials, attributable to the depolarizing action of ACh. In myasthenic patients this was followed by a period of transient potentiation of induced potentials. In both normal subjects and myasthenic patients there then ensued a late prolonged depression of induced potentials, attributable to choline released as a result of hydrolysis of ACh. This late depression has the properties of a depolarizing block in normal subjects and a competitive block in patients with myasthenia gravis. These observations indicate that the defect in neuromuscular transmission in myasthenia gravis may be due to a competitive (ACh inhibitory) block produced by choline released in a normal manner during neuromuscular transmission following hydrolysis of endogenous ACh.

Neuromuscular Transmission in Myasthenia Gravis*

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It is now generally accepted that the functional abnormality in myasthenia gravis is a failure of neuromuscular transmission. Previous theories as to the causation of this disease have been based either on the unproved existence of a curare-like substance or on the contradictory results of studies of the action of acetylcholine in normal and myasthenic subjects. The introduction of the decamethonium salts (C 10), which act in a similar manner to acetylcholine,[1,5] offered a unique opportunity for re-investigation of this controversial matter.

In our studies we used a combination of volitional and electromyographic technics following intravenous injection of decamethonium iodide in both normal and myasthenic subjects.[2] The response of the various muscle groups throughout the body could thus be measured.

In normal subjects a dose of 2.5 mg. of decamethonium produced a 70 to 80 per cent reduction in the height of the action potential of the hypothenar muscles on supramaximal stimulation of the ulnar nerve and resulted in some muscle weakness. A subsequent intravenous injection of an anticholinesterase compound, tensilon,® at the height of the decamethonium paresis led to a profound increase in the degree of generalised weakness. In other words, injection of a neostigmine analogue in the presence of decamethonium paresis leads to potentiation of the depolarisation block.

Localised Myasthenia Gravis. In myasthenic subjects a completely different type of response was encountered. In those patients in whom the disease was localised to a few muscle groups (localised myasthenia gravis) and particularly in those in whom the symptoms of the disease were minimal, large doses of decamethonium could be tolerated without any evidence of a reduction in the height of the action potential or in volitional activity. For example, in a twenty-one year old woman, the injection of 10 mg. of C 10 failed to produce any detectable signs of paralysis, yet as little as 3 mg. is sufficient to produce extreme weakness in normal subjects under similar conditions. Furthermore, over 60 per cent of the dose injected into this myasthenic patient could be recovered from the urine and could be shown to possess potent depolarising activity when injected into the neck vein of the chick.[4] This resistance to the depolarising action of decamethonium was found to be present both before and six months after surgical removal of the thymus. Patients showing such marked tolerance to decamethonium are rare; there is little doubt that if further doses were given the patient would ultimately show signs of muscular weakness.

Generalised Myasthenia Gravis. In those patients in whom the myasthenic weakness was generalised (generalised myasthenia gravis) this apparent resistance to the depolarising action of C 10 previously described in the localised type was much less marked. The first muscles to show signs of paralysis due to the C 10 were always those which were clinically weak. When decamethonium finally brought about a neuromuscular block in the muscle of the myasthenic subject this block was not of the depolarising type but was a non-depolarisation (competitive inhibition) block. Evidence for this was that the block could readily be reversed by neostigmine or tensilon, was greater for tetanus than for twitch and also was unaccompanied by muscle tightness and fasciculations seen prior to depolarisation block. Nevertheless the brief improvement in muscle strength which was seen electromyographically in some myasthenic subjects was taken as evidence of depolarisation preceding onset of non-depolarisation block.

COMMENTS

These observations on patients with myasthenia gravis after injection of decamethonium

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suggest that the abnormality is one of an alteration in the response of the motor endplates, namely, a dual response (that is, depolarisation followed by non-depolarisation block).[3,4] Somewhere in the transition from the pure depolarisation response of the normal subject to that of the dual response which is best seen in the clinically weak muscles of myasthenic subjects there must be an intermediate position in which the endplates are resistant to depolarisation. The non-weak muscles of myasthenic subjects show this type of response which is best seen, therefore, in cases of localised myasthenia gravis.

The fact that these patients only have a few muscles showing clinical weakness, yet are able to tolerate large doses of intravenous C 10 without paresis, suggests that in myasthenia gravis the alteration in the response of the motor endplate resists depolarisation and, finally, the threshold for excitation is raised so that a non-depolarisation block ensues. Clinically the presence of resistance to the depolarising activity of C 10 can be used as a test for the presence of myasthenia; this has been found particularly useful in differentiating hysterical paresis from that due to localised myasthenia.

It is important to stress that the clinically weak muscles of the myasthenic are the first to be affected by decamethonium. If the myasthenic weakness was due either to a circulating or locally produced curare-like substance, then one would expect these muscles to be the most resistant to the depolarising activity of decamethonium because the two types of block are antagonistic. Such is not the case. Our findings with decamethonium are inconsistent with such a hypothesis and can best be interpreted on the basis of the dual response.

A dual mode of response to decamethonium identical to that observed by the authors in myasthenia gravis has been described as occurring in various species of animals.[5] Thus while cat, avian and frog muscle respond to decamethonium by pure depolarisation, the muscles of monkeys, dogs and hares can exhibit features of both depolarisation and non-depolarisation block with this substance.

Although there is no direct evidence as yet that the motor endplates in man respond to acetylcholine or one of its breakdown products in this dual manner, such a conception would account for all the clinical features of myasthenia gravis.

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Comparison of Simultaneously Recorded Electrical and Mechanical Activity in Myasthenia Gravis Patients and in Partially Curarized Normal Humans*

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OBJECTIVE testing of muscle function has been performed in myasthenia gravis patients by means of mechanograms and electromyograms.[1-4] To date none of these studies has utilized simultaneous recording of tension and action potentials although discrepancies between electrical and mechanical activity were pointed out by Harvey and Masland in 1941.[1] In myasthenia gravis patients these workers were unable to demonstrate any alteration from normal in the action potential of a single maximal induced twitch although they did find a progressive decline in amplitude of successive action potentials with repetitive stimulation at rates below 40/sec. On the other hand, Lindsley[2] found that the tension developed by single twitches in myasthenics was less than normal and this single twitch tension increased with the administration of neostigmine. Furthermore, Odom, Russel and McEachern,[3] and Pritchard[4] were unable to demonstrate any alteration in tetanus tension in myasthenia gravis patients unless stimulation rates faster than 80/sec. were used. The present paper is an attempt to resolve these views by measurement of simultaneous electrical and mechanical activity of skeletal muscle in myasthenia gravis patients and in partially curarized, otherwise normal, subjects. The results indicate the following: (1) tension studies are more accurate indices to the degree of muscle weakness than the electromyogram and (2) there may be changes in the contractile ability of the muscle in these two conditions at a time when no evidence of neuromuscular transmission block can be observed.

Apparatus and Method. These have been described in detail in a previous paper.[5] Figure 1 is a diagram of the apparatus we used. After suitable splinting, the thumb was placed in a stirrup which was attached to an overhanging metal bar to which strain gauges were bonded. When the thumb was adducted, the bar was bent very slightly and the strain gauge output increased, causing a deflection of one beam of a double-beam cathode ray oscilloscope. The other beam of the oscilloscope was deflected by action potentials picked up from a surface electrode on the belly of the adductor pollicis

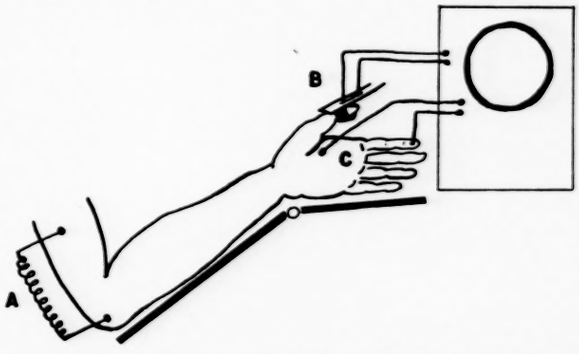

FIG. 1. Apparatus: A, stimulator; B, strain gauge to measure tension; C, electrodes to pick up action potentials. Both A and B are recorded on dual-beam cathode ray oscilloscope, the screen of which is photographed on 35 mm. film moving continuously at the rate of 1,200 in./min.

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muscle. The short adductor of the thumb was induced to contract by supramaximal percutaneous stimulation of the ulnar nerve at the elbow.

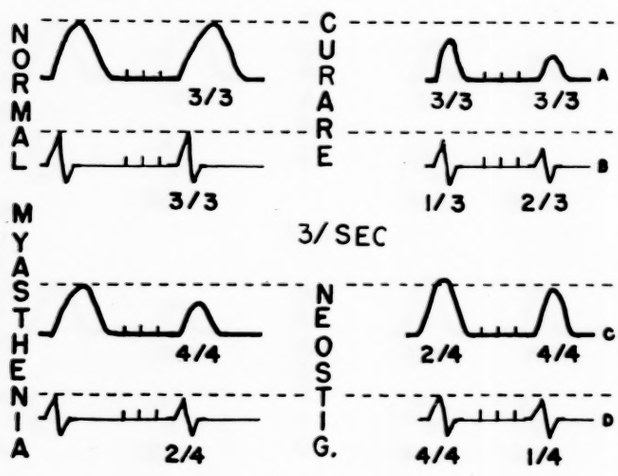
RESULTS

Figure 2 shows the results with 3/sec. stimulation. No fusion of successive single twitch tensions occurred at this stimulation rate either in the myasthenia gravis patients or in the normal subjects. Therefore, with 3/sec. stimulation one must note: (1) the initial single twitch and (2) the pattern of the successive single twitches. There was no difference between the tension and the action potential amplitude of the initial single twitches of normal subjects and myasthenia gravis patients. No significance can be placed on the finding that the mean initial single twitch tension was somewhat less in the myasthenics because no attention was paid to initial muscle length from individual to individual and the groups were not homogeneous as to age and sex. However, while the tension of successive twitches did not decrease in all the normal subjects, it did decrease significantly in all the myasthenics so that the tension developed by the fifth twitch was less than that developed by the first in all the myasthenics. There was a discrepancy between the tension and the electromyogram because the decrease in tension of successive twitches at 3/sec. was accompanied by a decrease in action potential amplitude in only two of the four myasthenia gravis patients.

In the three normal subjects after the intravenous administration of *d*-tubocurare there was a decrease in the initial single twitch tension with a further decrease in the tension of successive twitches at 3/sec. In this instance there again was apparently no correlation between the electrical and mechanical activity of the muscle since in only one of the three subjects was there a decrease in the amplitude of the successive potentials.

After the administration of neostigmine there was a slight but significant increase in initial single twitch tension in two of the four myasthenia gravis patients, and in all four patients the decrement in successive twitch tensions was less marked than it had been before the drug was given. Again there was no correlation between electrical and mechanical activity since none of the myasthenics showed an increase in initial action potential amplitude and in only one of the four was there less marked decrement in the amplitude of successive action potentials after neostigmine.

Figure 3 shows the results with 30/sec. stimulation for one second. Complete tetanus occurred at this stimulation rate. Tension within the normal range developed in all four myasthenics



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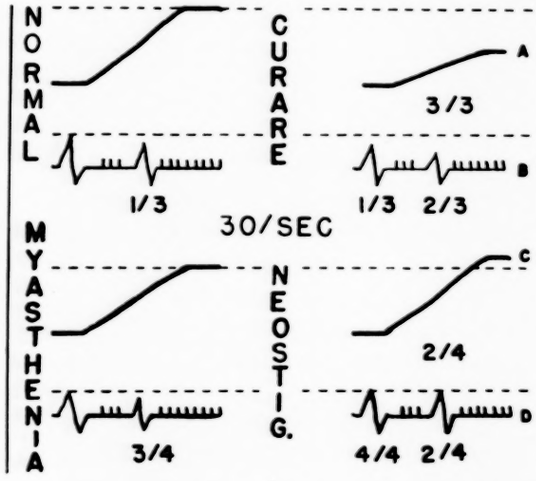

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FIG. 2. Three per second stimulation: A and C, tension-time curves of first and fifth twitches; B and D, simultaneously recorded action potentials. Time relationships of tension-time curves and action potentials are not accurate in this and subsequent figures. In this and subsequent figures, the ratio equals number of subjects showing a given pattern/total number of subjects.

FIG. 3. Thirty per second stimulation: A and C, tension curves of beginning of tetanus to plateau; B and D, simultaneous first and fifth action potentials.

and three of the four showed a significant decrease in the amplitude of successive potentials. (However, one of the normal subjects showed a similar electromyographic pattern.) After neostigmine two of the three myasthenics who had had an abnormal electromyogram before the drug showed a significant increase in developed tension. In these two patients the successive action potentials were sustained better than they had been before neostigmine. After neostigmine there was no significant change in tension in the other patient who had had an altered electromyogram before the drug even though this pattern was restored toward normal.

After intravenous *d*-tubocurare less tension with 30/sec. stimulation developed in all three normal subjects. On the other hand, only one of the three showed a decrease in initial potential amplitude and only two of the three showed a decrease in amplitude of successive potentials, which was not present prior to the administration of the drug.*

To recapitulate, in myasthenia gravis patients and in partially curarized otherwise normal subjects mechanical activity can be altered without a corresponding change in electrical activity. This probably means that alterations can occur in the contractile processes of muscle in these two conditions apart from any change in neuromuscular transmission. It is apparent from the results of this study that changes in tension are more accurate measures of muscle weakness than are changes in the action potential. The method of recording simultaneous mechanical and electrical activity with indirect supramaximal stimulation of the muscle appears to be an excellent objective measure of the effects of muscle relaxant drugs in man. This method eliminates many of the objections which have been raised to dynamometer methods of testing muscle strength.[6] If a choice between mechanical and electrical activity must be made, we believe that the recording of mechanical activity would be more useful, more accurate, more convenient and less expensive.

Rather than speculate at this point upon why a difference should exist between electrical and mechanical activity, we would like to present some effects of tetanus upon the single twitch in myasthenia gravis patients and in partially curarized, otherwise normal individuals. (Fig. 4.) It can be seen that in normal subjects a single twitch following a tetanus of 30/sec. for one second develops greater tension than a pretetanus single twitch. This normal post-tetanic potentiation of twitch tension is unaccompanied by any change in the action potential and lasts

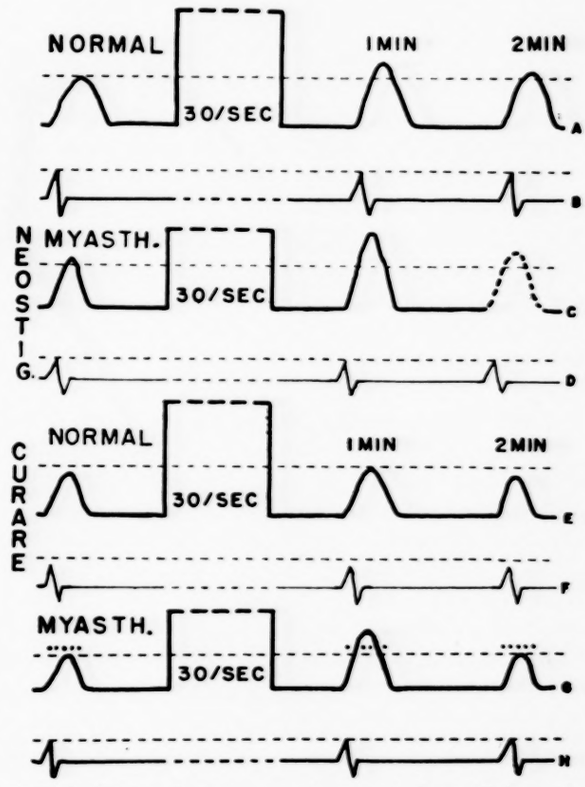

FIG. 4. Effects of tetanus on single twitch: A, C, E and G, tension-time curves of single twitch, tetanus of 30/sec. for one second, and single twitch at one and two minutes post-tetanus; B, D, F and H, simultaneous action potentials of single twitches. Dotted lines in G indicate the tension developed by the pretetanus twitch after neostigmine.

at least ten minutes.[5] In the myasthenia gravis patients and in the partially curarized normal individuals we found that post-tetanic potentiation of twitch tension did occur but did not last as long as it did in normal uncurarized subjects. We had expected to find that the post-tetanic potentiation of twitch tension in myasthenia gravis patients and partially curarized individuals would be accompanied by an increase in the dimensions of the action potential (amplitude, duration and area), indicating that more fibers were active as a result of the restoration of neuromuscular transmission by the tetanus.

* It is of interest to note that the individual who showed a decrease in tension but not in electrical activity at both 3/sec. and 30/sec. stimulation after curare had marked symptoms of weakness of laryngeal and pharyngeal muscles.

However, this was not the case since the post-tetanic potentiation of twitch tension was not accompanied by changes in the action potential dimensions. These findings suggest that in myasthenia gravis patients and in partially curarized normal subjects an alteration in the contractile ability of skeletal muscle can account for muscle weakness at a time when there is no alteration in neuromuscular transmission. We do not mean to imply that the myasthenic is identical to the curarized subject, for further analysis of the post-tetanus state shows that this is not so. These data will be reported elsewhere.

When neostigmine was administered to the myasthenia gravis patients we found that post-tetanic potentiation of twitch tension occurred, lasted as long as in normal subjects and was still unaccompanied by changes in the action potential. We interpret these findings to mean that neostigmine can restore muscle strength in myasthenia gravis patients by altering the contractile processes without causing any observable change in neuromuscular transmission.

Post-tetanic potentiation of twitch tension may in part offer an explanation for the fact that at 30/sec. stimulation some myasthenics show normal developed tension despite a progressive decline in action potential amplitude. It is possible that during the course of the tetanus some muscle fibers may alternate between activity and inactivity. When they are in the inactive phase following activity, they are in the post-tetanus state. Then, when they again become active they contribute a greater increment of tension than they would have had they continued to be active throughout the tetanus. This view has been suggested by Brown and Burns[7] to explain similar findings in fatigued cat skeletal muscle.

SUMMARY

In summary, the present study of simultaneous electrical and mechanical activity in myasthenia gravis patients and in partially curarized normal subjects indicates that tension changes at slow (3/sec.) and moderate (30/sec.) stimulation rates are more accurate indices to muscle weakness than changes in the action potential. In addition, the results in these two groups of subjects suggest that the weakness may, at least in part, be due to changes in the contractile ability of the muscle apart from any change in neuromuscular transmission. Neostigmine apparently is capable of restoring the contractile ability of muscle at a time when no alteration in neuromuscular transmission can be observed.

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The Thymus and Myasthenia Gravis*

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THE notion is not a new one that the thymus might have something to do with myasthenia gravis; this is obvious from publications on the subject since the beginning of this century. Furthermore, it is clear that interest in the experimental investigation of the subject has arisen and has been maintained as a result of consistent clinical observations on patients with myasthenia gravis. The idea of trying to produce a myasthenic animal or at any rate of attempting to reproduce the essential features of this condition in the whole or part of an animal has occurred to many, but the results of such attempts as have been published are conflicting and in general have provided little evidence that the thymus influences muscular function. I shall not comment on the results of implanting pieces of thymus gland other than to draw attention to the fact that the duration of effect was transient, and that in no case has the tissue been transplanted to ensure vascular anastomosis with the recipient.

I would like briefly to summarise the published evidence regarding the effects of thymus extracts, for statements are often made about findings by one author not being confirmed by another, when in fact the experiments have not been repeated. In most instances calf thymus extracts have been used; a few extracts have also been made with thymus glands removed from patients with myasthenia gravis. The extracts of calf thymus have usually been made with saline solution and tested in doses equivalent to 0.4 to 20 gm. of fresh thymus. The biologic preparations used to assess the activity of the extracts have been of several types; the method has usually been to produce muscular weakness or fatigue in the unanaesthetised animal or in a nerve-muscle preparation by stimulation of the nerve supplying a muscle or group of muscles, and to observe whether the extract prevented, relieved or induced muscular fatigue.

Adler produced in the dog rapid fatigue of the anterior tibialis muscle in response to repeated indirect tetanic stimulation, after intravenous injection of an extract equivalent to 4 gm. of fresh calf thymus. This effect lasted for about twelve hours and was relieved and prevented by neostigmine. When the extract was injected subcutaneously the effect was delayed in onset but lasted for about twenty-four hours. Extracts of calf spleen did not produce these effects. Klose and Vogt used pressed thymus juice in doses equivalent to 10 to 20 gm. of fresh thymus. When this was injected intravenously or subcutaneously into thymectomised puppies it aggravated the signs of fatigue already evident in these animals. Normal dogs were unaffected. Constant and his colleagues observed, in the cat, depression of the muscle twitch in response to stimulation of its nerve after a saline extract equivalent to 2 gm. of dog thymus was injected into the carotid artery.

A number of attempts have been made to extract thymus glands from myasthenic patients and to test them for curare-like activity. The only evidence of a positive nature is that by Constant and his colleagues who observed depression of the muscle twitch after intracarotid injection of a saline extract equivalent to 1 to 2 gm. of fresh thymus gland.

Choice of Solvent, Biologic Test and Standard. The negative results reported in many of these investigations are probably due to the use of relatively insensitive biological tests and to insufficient doses of thymus, for an estimate of the amounts of tubocurarine required to produce an effect on these test objects shows that each dose of thymus would require to contain curare-like activity equivalent to 0.1 to 1 mg. *d*-tubocurarine. This is beyond the usual total activity obtained from one myasthenic thymus gland. McEachern, at least, was aware of some of these difficulties when he briefly reported his results in a masterly review of the subject ten years ago.

Our first extracts were prepared from myasthenic thymus glands and consisted of prelimi-

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nary extraction with acetone followed by saline extraction of the acetone-soluble residue and of the acetone-dried gland. The combined saline extracts were adjusted to the pH of Tyrode solution. The use of acetone in the extraction process was fortuitous, for it was primarily used for the storage of the thymus glands after their removal from patients. In view of the variations in water and fat content of fresh thymus, the activity of all extracts is referred to a weight of acetone-dried thymus, 1 gm. of which is equivalent to 5 to 7 gm. of fresh thymus gland.

For estimating the activity of the extracts we used the isolated rat phrenic nerve diaphragm preparation described by Bülbring (1946), which is more sensitive to tubocurarine than any of the tests previously described. With slight modifications we were able to detect a depression in the muscle twitch response to as little as 2 μg. of tubocurarine. The selection of *d*-tubocurarine as a standard substance with which to compare the action of thymus extracts was based entirely on the convenient assumption that if any effect of thymus extracts was related to the clinical features of myasthenia gravis, it would approximate more closely to the action of this neuromuscular blocking agent than to any other drug known at that time. Subsequent experiments have shown that the actions of thymus extracts differ in a number of ways from those of *d*-tubocurarine.

Preliminary Results. Biologic assay of human thymus glands obtained at operation from myasthenic patients and at autopsy from non-myasthenic infants and adults showed a wide variation in the activity that can be extracted from the thymus. The results show that the most active extracts were of myasthenic glands whose removal produced the greatest therapeutic benefit and also that some of the infant thymus gland extracts were more active than those of normal adults.

It was now obvious that if these results were related to a function of the thymus, the amount of activity secreted daily from the gland must greatly exceed the amount that can be extracted from it, for the most active myasthenic thymus extract had a total activity equivalent to ¼ mg. of *d*-tubocurarine not sufficient to produce any effects in man. Large batches of thymus glands hence would be required for preparation of extracts; since it was unlikely that these would be obtained in sufficient amounts from myasthenic patients, animal thymus glands should be obtained from very young animals and preferably from foetuses. This is a point of some importance, for in later work with calf thymus extracts we were unable to explain some conflicting and variable results until we found that the Ministry of Food officials, who at the time controlled the supply of material from the abattoir, did not distinguish between young calves and bullocks two to three years old.

Later Methods of Extraction. Thymus extracts prepared in this way are unstable in aqueous solution and lose at least 50 per cent of their activity when stored for twenty-four hours in the refrigerator. They are quickly inactivated by boiling in acid or alkaline solution and a solution of the ashed residue is inactive. Attempts were made to prepare a more stable extract by freeze-drying the aqueous solution. The freeze-dried residue is a buff-coloured amorphous powder, readily soluble in water, 1 mg. of which was equivalent to 0.5 gm. of acetone-dried thymus. Ether and petroleum ether were found to remove only inactive fatty material.

Considerable difficulty arose regarding the processing of large batches of freeze-dried material and the original process of acetone and saline extraction was reinvestigated. When fresh thymus was shaken twice with acetone most of the activity was removed by the acetone and our subsequent method of extraction consisted in concentrating the filtered acetone extract under reduced pressure to a small volume. This concentrate when centrifuged separated into three layers, the lowest of which was brown. The brown layer so obtained is a viscous liquid which when concentrated *in vacuo* yields a brown residue which is readily soluble in water and in saline solution, forming an amber-coloured solution. The brown layer appears to be stable when mixed with acetone and kept in the refrigerator, but aqueous solutions of it at room temperature are unstable.

Batches of foetal whale thymus, human infant thymus and calf thymus, human lymph node and human voluntary muscle have been extracted in this way. The yield of brown layer has been greatest from foetal whale thymus and least from calf thymus. No definite brown layer separated from human lymph node and human voluntary muscle.

Mode of Action. A general study of the actions of thymus extracts has not yet been made; attention has been focussed primarily on their effects on neuromuscular transmission and

voluntary muscular movements by comparing the actions of thymus extracts with those of *d*-tubocurarine and decamethonium (C 10).

Frog Rectus. The response of the isolated frog rectus muscle to acetylcholine is decreased in the presence of tubocurarine and increased by C 10. There is a marked resemblance between the effects of myasthenic thymus gland extract and that of C 10, in which small doses potentiate the action of acetylcholine and larger doses produce a prolonged contracture.

In the initial stages of the investigation this result came as a suprise but was always obtained under the same conditions. The possibility was considered that the extract might contain either one substance with two different types of action, or two different substances. To explore this the freeze-dried material was extracted with alcohol; the alcohol-soluble fraction produced an effect similar to that of *d*-tubocurarine but recovery from the former was more delayed than from the latter.

The precise conditions necessary for the separation of this apparent curare-like fraction have not been determined, for although several attempts were made to repeat this separation from extracts of myasthenic thymus gland they have not always been successful. Furthermore, we have so far failed to obtain a similar fraction from extracts of calf thymus. In all cases the alcohol-insoluble fraction has always increased the response to acetylcholine.

Antagonism with Anticholinesterases. It was an attractive proposition to determine the action of thymus extract in the presence of eserine. The curare-like effect on the frog rectus preparation was not reduced when eserine was added to Ringer's solution, nor was the response of the rat diaphragm preparation to the extract altered in the presence of eserine. The effect of myasthenic thymus extract is not reversed, as is typical of tubocurarine. Similar results have been obtained with foetal whale thymus extract on the cat tibialis/sciatic nerve preparation.

Effects on Response to Direct and Indirect Stimulation. Some experiments were planned to study the effect of thymus extract on the response of the isolated rat diaphragm preparation to indirect and to direct stimulation. It was hoped that the results so obtained would determine whether the action resembled that of tubocurarine more closely than that of C 10. This, of course, is not a critical method of distinction.

When more adequate amounts of foetal whale thymus extract became available by the simplified acetone extraction, the effect of brown layer was observed on the cat tibialis preparation stimulated through its nerve. Small doses of the extract produce an increase in twitch height. Larger doses produce a transient contracture followed by a depression in twitch height. These effects closely simulate those of C 10. The response, however, was not entirely typical of a depolarising effect since tetanus was not sustained.

When the chronically denervated cat tibialis muscle is stimulated directly, the response to thymus extract consists of a contracture similar to that observed after an injection of C 10.

Action on Chicks. Another approach was made by injecting brown layer intravenously into young chicks. Buttle and Zaimis have shown that in this way C 10 produces a rigid extension of the limbs and retraction of the head with a typical spastic paralysis; by contrast, after tubocurarine there is flaccid paralysis and the neck is flexed.

Within five seconds of a dose of 5 mg./gm. of body weight of foetal whale thymus extract the chicks show initially extension of the limbs, fluttering of the wings and irregular respiration, with loss of cheeping; this is followed within a minute by a flaccid paralysis of the limbs and flexion of the neck lasting for five to eight minutes, thereafter the cheep is restored and the chick recovers and walks about within ten to fifteen minutes. Larger doses caused rapid respiratory paralysis and the chicks died within forty-five seconds. Extracts of human lymph nodes and of voluntary muscle when similarly injected had no effect.

In view of the limited amounts of foetal thymus extract, only a few observations were made on the mouse. A modification of the righting reflex test (described by Collier and his colleagues in 1949) was used. The mouse is placed on a revolving drum rotating on a horizontal axis. A positive effect is scored if during a three-minute period the animal fails to remain on the drum. After an intravenous injection of tubocurarine (0.1 μg./gm.) the mice fall from the drum in fifteen to forty-five seconds and usually recover within five minutes. Foetal whale thymus extract in doses of 5 mg./gm. intravenously caused the animals to fall off within one and a half to two minutes; they usually recovered in eight to ten minutes. Larger doses

(7.5 to 25 mg./gm.) caused general paralysis of limbs and of respiration.

While these experiments on unanesthetised animals lend support to the observations made on isolated tissue and other nerve muscle preparations, it is clear that the nature of the action produced by thymus extract does not comply with the criteria demanded of a neuromuscular blocking substance of either a competitive or a depolarising type.

Effect of Thymus Extract on Response to Tubocurarine. Since all the evidence so far obtained indicates that the action of the extract is transient, and at most lasts for only about fifteen to twenty minutes, it was concluded that the prospects of maintaining a state of muscular paralysis in animals could probably be brought about only by frequent injections of extract. Until sufficient supplies of thymus extract became available it was decided to determine whether thymus extract modified the response of the nerve-muscle preparation to neuromuscular blocking drugs, as appears to be the case in patients with myasthenia gravis.

In the cat tibialis preparation thymus extract appeared to increase the sensitivity of the preparation to tubocurarine, an effect not observed with lymph gland extract. Observations on the chick showed that when tubocurarine was given within eighteen minutes of an injection of foetal whale thymus extract the dose required to produce typical flaccid paralysis was only one-quarter of that normally required. When the time between the injection of thymus extract and tubocurarine exceeded twenty minutes, increased sensitivity was not observed.

CONCLUSIONS

No far-reaching conclusions can be drawn from these results for the work has only reached a preliminary stage. There is an immediate need to purify the thymus extract and to determine whether the effects which have been observed are due to one or to more than one active substance. At present, work is in progress on this aspect.

It is not possible to state with certainty that these effects are peculiar to thymus gland extracts but they have not been observed with extracts of lymph nodes and of voluntary muscle. The effects described have been most readily obtained with foetal whale thymus gland and myasthenic thymus gland extracts; the effects seen with calf thymus extracts have been more variable and we do not know whether this is due to quantitative or qualitative differences. Within certain limits of doses used in these experiments the action of thymus extract is transient and the nerve-muscle preparation or unanaesthetised animal recovers spontaneously. The effect is not reversed by neostigmine but it is not known what changes, if any, result from repeated administration of the extract.

Thymus extracts do not produce, in the test objects used, all the characteristic features of myasthenia gravis. The facts so far elicited, however, are not inconsistent with the view that the thymus gland may release or be intimately concerned with the release of a substance which affects neuromuscular transmission.

This assumption has the merit of affording an explanation for the remission of symptoms, on the grounds of a temporary cessation or reduction in the amount of substance released. Whatever the nature of its action it appears, up to a point, to be a reversible one, and in these circumstances removal of the thymus effectively controls the condition.

On the other hand, if the substance is released over a prolonged period, as in patients who have had the disease for many years, its action may then result in irreversible changes at or near the neuromuscular junction which are not influenced by removal of the thymus even though the response to neostigmine is unaltered.

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DISCUSSION

DR. J. ALFRED RIDER: The inhibiting effects of extracts of thymus glands on the rat phrenic nerve-diaphragm preparation as reported by Professor Wilson are of great importance and of unusual interest to all who believe that the thymus gland may play an etiologic role in myasthenia gravis. There are two points in his study which should be re-emphasized: First, the degree of activity of the myasthenic gland in these experiments bore no direct relationship to the severity of the myasthenia gravis as judged by the

neostigmine requirement; second, a very definite correlation was shown between the activity of the thymus glands of myasthenic patients and the beneficial results obtained following thymectomy. If this correlation holds up in further experiments it might be taken to indicate that an inhibiting substance is present in the thymus glands and possibly in other organs of the body or in the lymph nodes as well. Thus in cases in which sufficient amounts of the inhibiting substance were being concentrated or produced in the thymus, thymectomy would be warranted. In patients in whom this condition did not prevail the operation would probably be of no benefit. The etiologic and therapeutic implications are therefore important.

The results of my own experience with thymic extracts and serum from patients with myasthenia gravis have not been as definite or as clear-cut as those of Professor Wilson. The first experiments were begun in 1949, in collaboration with Drs. Constant, Porter and Andronis. Tissue extracts were injected into the carotid artery of cats prepared as described by Porter and Wharton.[1] With this method the brain and cord of the cat is pithed, and the animal is maintained by artificial respiration. The tibialis anticus muscle is used to record muscular contractions. The peroneal nerve, with its blood supply intact, is cut and drawn into a glass tube electrode; branches of the peroneal nerve to other muscles are severed. The nerve is stimulated once every three seconds by break shocks from a large inductorium. When the contractions have been regular for several minutes, 5 to 10 cc. of the extract to be tested are injected into the carotid artery. The extracts used were made by emulsifying the organs in a 1 to 5 dilution of normal saline for a period of several minutes and passing the emulsion through a glass-wool filter. The resultant filtrate was employed in all determinations.

In seven experiments using extract of myasthenic thymus removed surgically, diminution in muscular contraction occurred in five instances. Results in two tests were doubtful. In three experiments using extract of myasthenic thymus removed at autopsy, muscular contraction was doubtful in one instance and positive in two. This means that there was decrease in muscular contraction which began in two or three minutes and persisted for several minutes. In four experiments with extracts of non-myasthenic thymus removed at autopsy, diminished muscular contraction occurred in each case. Two experiments using extracts of spleen removed from the same patients increased the height of muscular contractions in each case. In all but one of the experiments in which the muscular contractions were reduced, recovery of the contraction height took place after several minutes. The data from these experiments would seem to indicate that saline extracts of the thymus gland, whether derived from normal subjects or from patients with myasthenia gravis, contain a substance capable of reducing neuromuscular response to a given stimulus.

More recently I performed a different group of experiments in an attempt to demonstrate the inhibiting effects of myasthenia gravis serum, as well as extracts of normal and myasthenic thymus gland, upon a rat nerve-muscle preparation. The technic used was a modification of that described by Ingle.[2] Young adult white rats of the Sprague-Dawley strain, male and female, adrenalectomized and non-adrenalectomized, were used as test animals. These animals were anesthetized with nembutal,® 35 mg./kg. of body weight, given intraperitoneally. The jugular vein was canalized for the injections. The sciatic nerve was stimulated supramaximally with a direct electrical current at the rate of five per second, and the gastrocnemius contractions were recorded upon a kymograph. The serum to be tested was injected, undiluted, in amounts of 0.2 to 1 cc. The thymic extracts were either water or saline homogenates in a dilution of 1 to 5 filtered through a glass filter or alcoholic extracts evaporated to dryness and then redissolved in water or saline so that the final dilution represented a 1 to 5 concentration.

The results of thirty-eight such experiments were as follows: Four experiments using extracts of beef thymus and four experiments using extracts of pork thymus showed no effect upon neuromuscular response. In twelve experiments with extracts of thymus obtained from patients with myasthenia gravis at operation or autopsy a definite decrease in neuromuscular contractions occurred in four animals with no decrease in eight. In twelve experiments using myasthenia gravis serum a definite decrease in muscular contraction was noted in two animals, but in ten there was no decrease in contraction. Extracts of thymus gland removed from newborn infants at autopsy were injected into six animals; a decrease in muscular contraction was noted in two. Numerous injections with normal serum, saline and alcohol had no effect upon muscular contractions.

It must be emphasized that in all of the experiments in which muscular contraction was diminished there was a decrease and not infrequently a temporary complete cessation of respiration. When respiration was resumed there was obvious hyperpnea. Whether this effect resulted from inhibition of the muscles of respiration by the injected substance in a manner analogous to the inhibition produced on the gastrocnemius muscle, or whether this was part of an anaphylactoid reaction could not be determined. If the latter were the case, then the results reported might be

[1] Porter, E. L. and Wharton, P. S. Irritability of mammalian nerve following ischemia. *J. Neurophysiol.*, 12: 109–116, 1949.

[2] Ingle, J. D. The quantitative assay of adrenal cortico-hormones by the muscle work test in the adrenalectomized nephrectomized rat. *Endocrinology*, 34: 191–203, 1944.

of much less significance since the observed effects might have been only secondary and fortuitous.

It appears, then, that a substance is present in the serum and in the thymus gland of patients with myasthenia gravis which will decrease the muscular contractions in a mammalian nerve-muscle preparation. This effect, however, cannot be consistently demonstrated. Obviously, experiments of this type are difficult to perform. First, the amount of thymus gland available in any one case is small. Second, the effect of the glandular extract upon muscular contraction probably depends upon the strength or concentration of the inhibiting substance it contains. This, in turn, may or may not be related to the severity of the disease. It is certainly possible that negative results were obtained when a sufficient amount of material was not present in the thymus gland or when the amount of thymic extract injected was too small to induce the response. Then, too, in our experiments no real attempt was made to concentrate the presumptive inhibiting substance. Other tissue extracts and various precipitations should be investigated to see if it would be possible to concentrate this substance. Although these investigations show promise, it is apparent that a great deal of additional work remains to be done.

DR. SUMNER ZACK: Dr. Wilson's pioneering work on thymus extracts from myasthenic thymuses is well known.

We have been testing extracts prepared according to the acetone extraction method of Dr. Wilson (1952) on isolated frog sartorius muscles arranged in a chamber fitted with electrodes which permit direct and indirect stimulation. The isometric mechanogram was recorded with a strain gage. Acetone extracts prepared from thymus glands obtained from fifteen myasthenic patients, three spleens and two samples of striated muscle were tested.

Both acetone-soluble and acetone-insoluble fractions markedly decreased the muscle tension curve in two to twenty minutes. When the muscles became inexcitable on indirect stimulation, they also were inexcitable on direct stimulation. The active material is heat-stable, dialyzable and is antagonized by $CaCl_2$ added to the extracts.

Concentrations of potassium two to forty-four times the potassium concentration of frog Ringer's solution were found in the extracts. These concentrations of potassium are sufficient to produce the muscle inhibition observed.

More refined extraction procedures will be needed to find possible blocking activity in thymus extracts.

Present Status of Thymectomy in Treatment of Myasthenia Gravis*

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FEW medical papers have had the catalytic effect of that published in 1939 by Blalock and associates[1] describing apparent success in the treatment of myasthenia gravis by removal of a tumor from the region of the thymus gland. The paper was most timely and is generally credited with introducing the era of frequent thymectomy which, after a feeble beginning in 1941, became fully established in 1942. The paper was timely because by 1939 neostigmine and sulfonamide therapy had been available for only a short time; furthermore, thoracic surgery only recently had attained relative maturity. As a consequence of these developments a number of well trained and experienced thoracic surgeons were available and anxious to perform thymectomy on patients who had myasthenia gravis, and physicians, particularly those who were serious students of myasthenia gravis, had been sufficiently impressed by the gradual accumulation of evidence since 1901 of a relationship between myasthenia gravis and the thymus gland that they were willing to make available to the thoracic surgeons certain of their patients who had this disease.

Now, more than thirteen years after the era of frequent thymectomy began, the investigators who have attempted to determine the value of thymectomy do not present a united front with regard to its advisability in the treatment of myasthenia gravis. Naturally this failure to agree creates confusion among those who look to these investigators for guidance. The consensus of those students of myasthenia gravis in the United States who have not participated directly in the study of large series of patients undergoing thymectomy apparently was reflected by Westerberg,[2] who stated in effect that thymectomy evidently is not of great value or it would not be so difficult to determine whether or not it is helpful.

We believe that recently available data allow some clarification of the confusion regarding the value of thymectomy and allow for the conclusion that the operation is of value, at least for certain patients who have myasthenia gravis. Consequently we propose to review critically the more significant reports of recent years against the background of earlier contributions, compare them with newly prepared data from a study of our cases and prepare conclusions that will be of practical value in the selection of patients for thymectomy.

The first problem facing those who attempt to assess the value of thymectomy is to determine whether or not this surgical procedure influences beneficially the course of myasthenia gravis. The problem would be simple except for two factors. First, it was apparent early in the studies that not all patients undergoing thymectomy were improved as a result of operation. Second, it was well known that the course of myasthenia gravis could not be predicted precisely, since recovery or great improvement not infrequently occurred spontaneously. Thus improvement in any one patient after surgery could not be accepted as a result of thymectomy since the possibility of spontaneous improvement always existed. Naturally, if study of a relatively large number of thymectomized patients demonstrated that the course in this group differed sufficiently from the "usual course" of myasthenia gravis, a reliable decision as to the value of thymectomy could be attained. However, when we planned our study in 1941, at a time when the results only could be guessed at, this course appeared to us too hazardous, particularly when the "usual course" of myasthenia gravis was not known precisely. Of more promise in attaining a reliable result in which we and others might have confidence was the technic of using controls, whereby the course of a large number of patients with myasthenia gravis undergoing thymectomy could be compared

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with the course of a large group of such patients receiving only non-surgical treatment. We decided in favor of a controlled study, in spite of the great increase in labor and complexity entailed by the use of controls.

At about the same time studies to evaluate thymectomy were begun in a number of institutions in the United States having access to many myasthenic patients. In some centers, such as the New York Neurologic Institute[3] and the University of Michigan,[2] thymectomy was discontinued after a small series of operations had been performed because it did not appear to attain the desired result. However, further studies were done at Johns Hopkins University, the Massachusetts General Hospital and the Mayo Clinic. It is on reports of the relatively large series of cases from these institutions, particularly the ones in which controls were used, with comparison of the course in surgical cases with that in non-surgical cases, that we must lean most heavily for evaluation. For the most part, reports of single cases or of a small series of cases are of little help in view of the tendency to report favorable results and to refrain from reporting unfavorable ones. Consequently such reports have not been taken into consideration.

SUMMARY OF REPORTS EVALUATING THYMECTOMY

Keynes' Series. Keynes, a London surgeon, began early in 1942 to perform thymectomy on all myasthenic patients referred to him by physicians for that purpose unless he found some specific contraindication to surgical intervention. By 1954 he had amassed the largest series of thymectomies in the world. Under these circumstances no opportunity arose to develop a control series against which to compare the surgical results.

In a Hunterian Lecture given in 1945, Keynes[4] reported that he had operated on fifty-one patients for removal of the thymus gland and had assessed the results in thirty-three of the patients; he concluded that twenty of the thirty-three patients were greatly improved. By 1949, Keynes[5] had performed 155 thymectomies and had assessed the results in 120 patients subjected to removal of non-neoplastic thymus glands. The breakup into groups is as follows: (1) well, thirty-nine patients (32.5 per cent); (2) greatly improved, forty patients (33.3 per cent); (3) somewhat improved, thirty-one patients (25.8 per cent), and (4) no better, ten patients (8.3 per cent). Thus 65.8 per cent of these patients became well or greatly improved. Keynes reported the average age of group 1 as 28.3 years, of group 2 as 28.4 years and of group 3 as 32.1 years; he stated, "The influence of age on results is less important than at first appeared probable."

The average duration of myasthenia gravis at the time of surgery was two plus years for group 1, five plus years for group 2 and seven plus years for group 3. The average age of the patients in whom the results were classed as failures was nearly forty years, and the duration of disease tended to be long in these patients. Consequently, Keynes concluded, "It seems clear that a long history is prejudicial to recovery after operation."

Ross[6] in 1952 made an independent assessment of the results of removal of non-neoplastic thymus glands in one hundred myasthenic patients who had survived surgery by Keynes. The results were as follows: (1) quite well, forty-one; (2) greatly improved, twenty-six; (3) somewhat improved, twenty; (4) unimproved, six, and (4) dead, seven.

It is well to point out that Keynes uses the designation "quite well" as descriptive of patients who are quite well and require no neostigmine. They may be in complete remission or they may manifest minor evidences of myasthenia gravis, such as ptosis or diplopia or both. Consequently this group cannot be compared with our group in which results are designated "plus four, complete remission." If a minor degree of myasthenia gravis persists, we classify the results as "plus three, considerably improved."

By 1954,[7] when Keynes' series of surgically treated patients had reached "more than 200 patients without thymomas," his conviction regarding the value of thymectomy had not lessened and he stated in one of his conclusions that, "A large proportion of myasthenic patients are permanently and often completely relieved of their symptoms following thymectomy." Keynes has been supported by Sellors[8] of England and Dunlop[9] of Scotland who analyzed results after thirty-seven and nineteen thymectomies, respectively, and concluded that the operation was effective.

Johns Hopkins University Series. American workers have been cautious in attributing value to thymectomy and only in 1950 or thereafter did the investigators in this country publish

material indicating that they took a firm stand in favor of thymectomy.

Blalock and his associates,[1] in the aforementioned paper that precipitated the era of frequent thymectomy, said, "In concluding, we wish to emphasize again the absence of conclusive proof that the improvement noted in our patient is due to removal of the tumor from the thymic region." The second report of the Johns Hopkins University series was by the Blalock group[10] in 1941; it was concerned with six patients who underwent thymectomy, none of whom had a thymoma. Their early experience was summarized as follows: "The early results are encouraging and suggest strongly that the thymus gland is concerned in some manner in the genesis of myasthenia gravis."

In 1944, when Blalock[11] last wrote on his experience at Johns Hopkins University, he reported on twenty surgically treated patients, two of whom had thymomas. Of seventeen patients surviving surgery, one died later, three were little if at all improved, five were moderately improved, five were considerably improved and three were essentially well. Blalock's conclusions remained fundamentally conservative, although he said, "The early and sustained improvement which has been shown by some of the patients makes it difficult to escape the conclusion that thymectomy was at least partly instrumental in causing the alteration."

The next report of the Johns Hopkins University series was by Harvey,[12] in 1948. By that time thirty-two myasthenic patients had undergone thymectomy since 1941. Evidently the first case (1936) already mentioned, in which a tumor had been removed from the thymic region, had been excluded from the series, since complete thymectomy had not been attempted. Two of the patients were well and six were greatly improved. Harvey concluded that sufficient data were not yet available to allow final evaluation of the effect of thymectomy on the course of myasthenia gravis.

TABLE 1

MYASTHENIA GRAVIS

JOHNS HOPKINS UNIVERSITY SERIES[13]

Sex	Treatment	Patients	Average Duration of Disease (years)	Results (%)				
				In Remission	Improved	Unchanged	Worse	Dead
Male	None	41	7.2	17	12	12	15	44
	Thymectomy	19	7.2	22	16	21	0	42
	Irradiation	19	7.2	0	42	26	10	21
Female	None	77	9.0	14	22	22	12	30
	Thymectomy	25	9.7	12	36	24	0	28
	Irradiation	21	6.8	5	38	19	19	19

The last report of the Johns Hopkins University series was made by Grob[13] in 1953, at which time he presented the results in 202 patients with myasthenia gravis, forty-four of whom had been subjected to thymectomy and forty of whom had been treated by irradiation of the thymus. The results in this series are presented in Table 1. Grob's conservative conclusions are expressed in the following quotation: "The course of the patients who had thymectomy performed has been, in general, only slightly better than the course of those who had neither thymectomy nor irradiation. . . . A slightly higher proportion, particularly of the female patients, have improved, and fewer patients have become worse since thymectomy, but the difference is disappointingly small."

Massachusetts General Hospital Series. This series was first reported by Viets[14] in 1945 at which time it was composed of fifteen patients, four of whom had thymomas. Viets expressed his point of view as follows: "At the present time we are in the experimental stage."

Schwab[15] in 1949 expressed the opinion that thymectomized patients in the Massachusetts General Hospital series were progressing more favorably than would have been anticipated without thymectomy.

By 1950 Viets[16] had become convinced of the value of thymectomy in the treatment of myasthenia gravis. At that time his series numbered thirty-six patients and included seven instances of thymoma. The results in the twenty-nine patients who did not have tumors were summarized as follows: excellent, three; good to excellent, two; good, five; fair to good, two; fair, two; poor to fair, two; poor, two; dead, four, and too soon to estimate, seven. Viets did not present any data regarding specific controls and we interpreted his attitude as expressed in the main portion of his paper as being conservative in spite of his statement that "the results of thymectomy in the last nine years justify the continuation of the operation in patients with myasthenia gravis." However, an addendum to this report stated, "Since this paper was prepared, the report of Keynes (1949) on his large experience with thymectomy in myasthenia gravis has been published. Although he has approached the subject from a slightly different direction, our essential conclusions are identical. Both papers point out the value of thymectomy as a form of treatment for the disease."

Schwab and Passouant[17] in 1952 reported the results in forty-three patients who had removal of non-neoplastic thymus glands, excluding from analysis the seven thymomas in their series of fifty patients. Their results were as follows: completely cured, six; much improved, eighteen; little improved, ten; unimproved, five, and dead (operative deaths), four. Thus it was found that twenty-four of thirty-nine patients (62 per cent) who survived simple thymectomy were greatly improved, while the remission rate in fifty patients selected as controls was only 18 per cent. Unfortunately details regarding the selection of controls were not given in this report. Further analysis of their data indicated that the best results were obtained in young female patients.

TABLE II

MYASTHENIA GRAVIS

MASSACHUSETTS GENERAL HOSPITAL SERIES[18]

Results	Female				Male			
	Thymectomy		Control		Thymectomy		Control	
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
Complete remission	11	62	7	34	2	24	4	56
Considerably improved	22		11		4		10	
Improved	4	23	13	38	5	44	4	24
No essential change or worse	8		7		6		2	
Dead	8	15	15	28	8	32	5	20
Total	53	100	53	100	25	100	25	100

Schwab and Leland[18] in 1953 gave the last report of the Massachusetts General Hospital series; their data, which we consider of great importance, are summarized in Table II. Their study employed control patients who were matched with the thymectomized patients as to sex, age of onset, severity of disease and presence or absence of thymoma. The authors arrived at three important conclusions: (1) "The patients . . . who had thymomas . . . derived no evident improvement in their myasthenia from the operation." (2) "Males received no evident benefit from the operation. Thymectomy appears to be contraindicated in males in whom the onset of the disease was after 30." (3) "In females without thymomas, thymectomy appears definitely indicated."

Mayo Clinic Series. Our studies began in 1941 and the first patient of our series to have a thymectomy underwent surgery on December 20, 1941. A general survey of the series as it existed in November, 1954, is presented in Table III. Not all myasthenic patients studied at the clinic between November, 1941, and November, 1954, are included. The series has been

limited to unquestionable cases of myasthenia gravis that were studied by one of us (Eaton) except for a few surgical cases in which thymectomy was done by one of us (Clagett).

The first four papers concerning thymectomy in our series were published from August, 1943, to May, 1949.[19–22] Recognizing the pitfalls of drawing conclusions from too few cases studied for too brief a period, we refrained in these earliest reports from making any judgment with regard to the value of thymectomy. However, by 1949 it was considered that the studies had progressed to a point justifying conclusions. At the meeting of the American Neurological Association in June, 1949, we expressed our conclusions, which were published in 1950,[23,24] that controlled studies failed to support the opinion that thymectomy was of value in the treatment of myasthenia gravis. At that time adequate follow-up data were available on seventy-two of our seventy-five surgical patients and the results were compared with 142 control patients. The results among patients undergoing surgery were somewhat better than were those for the entire group of non-surgical patients, as is shown in Table IV.

TABLE III
MYASTHENIA GRAVIS
COMPOSITION OF MAYO CLINIC SERIES
(472 PATIENTS FROM NOVEMBER, 1941, TO NOVEMBER, 1954)

Categories	Female	Male	Total	
			Patients	Per cent
Surgical	72	49	121	26*
Thymoma	28	23	51	42†
No thymoma	44	26	70	
Non-surgical	186	165	351	74*
Thymoma	14	12	26	7‡
No thymoma	172	153	325	
Patients (total)	258	214	472	...
Thymomas (total)	42	35	77	16*
Thymomas (per cent)	16	16	.....	...

* Per cent of 472 patients.
† Per cent of 121 surgical patients.
‡ Per cent of 351 non-surgical patients.

However, in this study it became apparent at once that the patients selected for surgical treatment differed significantly from the remaining non-surgical patients. Furthermore it was apparent that some of the differences might well influence the statistical studies in favor of the surgically treated patients. For example, patients who were old or were extremely ill with myasthenia gravis or complicating diseases were excluded from the group to be treated surgically and relegated to the control group. Consequently it became obvious that control patients must be selected so that they and the patients undergoing surgery would be similar with regard to age and sex, duration and severity of myasthenia gravis, and duration of follow-up.

When such selection was done, the best case that we could make for thymectomy at that time was that a truly gratifying result of surgery (complete remission or considerable improvement) was obtained in 35.5 per cent of the patients surviving thymectomy as against a 28.5 per cent chance that a comparable result would occur without surgery. (Table IV.) This difference of only 7 percentage points did not appear significant. We were sufficiently discouraged with the results of thymectomy at that time that we temporarily ceased to offer it to patients except when thymomas were present.

Later that year in September, 1949, Keynes' second report[5] appeared and endorsed thymectomy more enthusiastically than had his first report (1946). In January, 1950, appeared the aforementioned report of Viets[16] that supported Keynes in concluding that thymectomy was of great value in the treatment of myasthenia gravis. Finally, as already indicated, Ross,[6] after his independent study of Keynes' cases, joined him in 1952 in attesting to the value of thymectomy. Needless to say, we were disturbed by the differences between our results and conclusions and those of the other workers.

Consequently we and Bastron made another evaluation of our series and reported our observations at the meeting of the Association for Research in Nervous and Mental Disease in December, 1952; they were published in the proceedings of that association almost a year later.[25] In this study of 1952 every possible factor that might influence results, such as age, sex, duration and severity of disease and duration of follow-up, was taken into account so as to procure as comparable a group of control patients as possible. Furthermore we realize that in our 1949 study the group of surgically treated patients was heavily loaded with those who had thymomas. We decided to eliminate them from the study. The gist of the 1952 study is presented in Table V, in which the results in forty-four

patients surviving removal of non-neoplastic thymus glands are compared with the results in forty-eight control patients. These findings indicated that the Mayo Clinic series did provide evidence for support of the value of thymectomy. By exclusion of the patients who had thymomas thymectomized patients became worse or died, whereas 29.2 per cent of the control patients were in these categories.

In order to compare our data with those of Schwab and Leland (Table II) with regard to the influence of sex on the prognosis after thy-

TABLE IV

MYASTHENIA GRAVIS

RESULTS IN SEVENTY-TWO SURGICAL AND 142 CONTROL PATIENTS

(MAYO CLINIC SERIES, 1949)[23,24]

Results	Patients (%)			
	Unselected		Selected	
	Surgical (72)	Control (142)	Surgical (62)	Control (56)
+4 Complete remission	6.9	7.7	8.1 } 35.5	8.9 } 28.5
+3 Considerable improvement	22.2	9.9	27.4 }	19.6 }
+2 Moderate improvement	20.8	10.6	24.2	14.3
± No essential change	27.7	43.0	29.0	39.3
− Worse	2.8	6.3	3.2	12.5
−4 Died	19.4*	22.5	8.1	5.4

* Six of the deaths (representing 8.3 per cent of the surgical patients) were the result of surgical treatment.

TABLE V

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON OF RESULTS IN SURGICAL AND NON-SURGICAL PATIENTS SIMILAR AS TO AGE, SEX, SEVERITY AND DURATION OF DISEASE

(MAYO CLINIC SERIES, 1952)

Results*	Surgical		Non-surgical	
	Patients	Per cent	Patients	Per cent
+4 Complete remission	8	18.2 } 50.0	5	10.4 } 29.1
+3 Considerable improvement	14	31.8 }	9	18.7 }
+2 Moderate improvement	9	20.5	7	14.6
± No essential change	9	20.5	13	27.1
− Worse	2	4.5 } 9.0	7	14.6 } 29.2
−4 Died of myasthenia gravis	2	4.5 }	7	14.6 }
Total	44	100.0	48	100.0

* Minimal follow-up study was three and two-thirds years.

it was found that removal of the non-neoplastic thymus gland resulted in a rate of remission of 50 per cent in contrast to a 29.1 per cent rate of remission among patients in the control group. It was also found that only 9 per cent of the

mectomy, we present in Table VI information regarding forty-seven of our forty-eight patients who had non-neoplastic thymus glands removed surgically and on whom follow-up data are available from our 1952 study. One patient in

whom accidental death occurred has been excluded. For comparison data are presented regarding fifty-four female and twenty-three male controls without thymomas who were comparable to the surgically treated patients in age, severity and duration of myasthenia gravis

TABLE VI

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON BY SEXES OF RESULTS IN SURGICAL AND NON-SURGICAL PATIENTS

(MAYO CLINIC SERIES)

Results	Female				Male			
	Surgical		Non-surgical		Surgical		Non-surgical	
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
+4 Complete remission	5	53	3	20	3	35	3	30
+3 Considerable improvement	11		8		3		4	
+2 Moderate improvement	6		8		3		4	
± No essential change	4		17		5		8	
− Worse	1		14		1		2	
−4 Died (surgical death)	1	13	..	33	2	18	..	17
−4 Died of myasthenia gravis	2		4		..		2	
Total	30	..	54	..	17	..	23	..

TABLE VII

MYASTHENIA GRAVIS WITHOUT TUMOR

COMPARISON BY AGES OF RESULTS IN THIRTY THYMECTOMIZED FEMALE PATIENTS AND FIFTY-FOUR FEMALE CONTROL PATIENTS

(MAYO CLINIC SERIES)

Results	Patients (%)			
	Less Than 30 Years		30 to 49 Years	
	Thymectomy (22 patients)	Controls (32 patients)	Thymectomy (8 patients)	Controls (22 patients)
+4 Complete remission or +3 considerable improvement	55	25	50	14
− Worse or −4 died of myasthenia gravis	14*	38	13	27

* Includes one surgical death.

and duration of follow-up. Our data are remarkably similar to those of Schwab and Leland in that they indicate good results for female patients but fail statistically to demonstrate surgery. Furthermore only 13 per cent of the female patients treated surgically became worse or died, whereas 33 per cent of the control female patients were in those categories.

In Table VII are presented data designed to determine whether or not older female patients, namely those from thirty to forty-nine years of age, do as well as younger ones, namely those less than thirty years of age. It would appear that the results in older women are not significantly different from those in younger female patients. Admittedly the small number of thymectomized women more than thirty years of age, namely eight in our series, prevents us from drawing definite conclusions with regard to this point but the data may be of value in reflecting trends.

It is not surprising that the sex of the patient should turn out to be a highly important determinant in prognosis after thymectomy. Pregnancy often appears to change the course of myasthenia gravis for better or worse, and it is the rule that aggravation of symptoms occurs during the days preceding menstruation. In all three of the aforementioned large American series females predominated among the younger group of patients and males among the older. In our series the patients who first noted symptoms of myasthenia gravis after the age of forty years were almost as numerous as those in whom the onset was before the age of forty. Females predominated 2:1 among the younger group and males predominated in roughly the same ratio in the older group.

Further discussion of thymectomy in the absence of thymoma will be resumed after consideration of patients in whom thymomas were present.

THYMOMA

In planning our study in 1941 for evaluation of thymectomy we decided to concentrate on patients in whom thymomas were present. This decision was influenced by the fact that the report of Blalock and associates[1] in 1939 and that of Campbell and co-workers,[26] presented at the meeting of the American Neurological Association in June, 1941, dealt with the favorable results achieved in two of three patients from whom thymomas were removed surgically. Furthermore at that time it was not illogical to presume that the removal of a large amount of thymic tissue would prove to be more effective than would removal of a small amount.

The first problem was one of roentgenologic detection of thymomas. We were unaware of any instance in which a roentgenologic diagnosis of thymic tumor had been made at the clinic prior to 1941. This was surprising in view of the fact that several hundred myasthenic patients had been examined roentgenologically. As a rule only stereoscopic films in the postero-anterior projection had been made. Abnormal shadows occasionally had been detected in the anterior mediastinum in patients who had myasthenia gravis but the roentgenologists had interpreted them as representing tumors other than those of thymic origin. This experience apparently agreed with that of other investigators. Viets and Schwab[27] in 1939 reported fifty cases of myasthenia gravis and stated, "We have been unable to associate myasthenia gravis with enlargement of the thymus gland. All roentgen ray studies were negative for enlargement of the gland." Viets in discussing the paper by Campbell and associates[26] 2 years later reported roentgenologic evidence of thymic tumor in only one of eighty-five cases.

Good[28] was challenged by the discrepancy between the frequency with which tumors of the thymus gland had been observed at necropsy and the infrequency with which they were found clinically. Consequently he undertook personal study of each of our myasthenic patients. In the first 206 consecutive patients studied a diagnosis of thymic tumor was made by roentgenologic methods in thirty-three, an incidence of 16 per cent. At last count the series had increased to 472 patients and the incidence of roentgenologic detection of thymomas had remained essentially the same. The incidence is equal in male and in female patients. The roentgenologic technic used at the clinic consists simply of use of stereoscopic films made in the postero-anterior projection followed by roentgenoscopy and usually by the exposure of films at the time of the latter examination, with the patient in the lateral or oblique projection. The accuracy of the roentgenologic diagnosis has been checked in 121 surgically treated patients and at necropsy in an additional twelve; it has been corroborated in all but two of these 133 patients. Consequently it is considered that the figure of 16 per cent is accurate to within at least a very few percentage points.

Grob[13] in the latest report of the Johns Hopkins University series stated that twelve of 202 patients had thymomas. In ten of these the course was rapidly fulminating and terminated in death regardless of whether or not thymectomy was performed. Among the forty-four surgically treated patients in Grob's series,

nine had thymomas; three of these nine died as a result of surgery and the remaining six died later of myasthenia gravis. Four patients of the non-surgical group who were found at necropsy to have thymomas had experienced rapidly progressive myasthenia gravis. Two other patients displaying roentgenologic evidence of thymoma have experienced a relatively benign course of many years' duration.

In the Massachusetts General Hospital series[18] seventeen thymomas had been encountered in seventy-eight surgically treated patients, an incidence of 22 per cent. Among the fifty-three surgically treated female patients, nine had thymomas; among the twenty-five surgically treated male patients, eight had thymomas.

Keynes[4] in 1946 reported that among his fifty-one surgically treated patients six had thymomas, one of which was malignant; two of these six patients died as a result of surgery and the others died subsequently of myasthenia gravis. Based on a study of these six patients, he stated, "Our experience suggests that when they [thymomas] occur the prognosis becomes very bad indeed."

In his 1949 report Keynes[5] eliminated patients who had thymomas from the assessment of results since "the course of the disease and the effect of operation in these patients [those who had thymomas] have been so different from the others. . . . " He went on to say, "The presence of a tumor implied a very much worse prognosis. Patients with tumors usually have a short history and severe symptoms which respond slowly and imperfectly to neostigmine."

In 1954 Keynes[7] stated that he had studied forty-one patients who had thymic tumors and that all those who had been treated surgically during the earliest part of the study had died. Of twenty-six patients who had been treated initially with roentgen rays, twenty had had the tumors removed subsequently with the following results: quite well and symptomless, four; considerably improved, eight; initial improvement, then relapse, three; no better, one, and died, four. Apparently on the basis of the results obtained in these twenty patients in contrast to the deaths occurring in all of the first fifteen patients subjected to surgery and who had not had preliminary radiation of the thymus, Keynes concluded, "The necessity for avoiding primary operation for tumors underlines the importance of carrying out a careful radiographic examination of every patient. . . . "

Data regarding fifty-five of our seventy-seven patients with thymomas on whom follow-up studies are available from our 1952 study are presented in Table VIII. For comparison Table IX presents a summary of results in all 312 patients comprising the 1952 study. The relatively poor prognosis for patients who have thymomas is reflected in these two tables. It is noted that 60 per cent of the patients who had thymomas (Table VIII) became worse or died, whereas only 39 per cent of the entire unselected group (Table IX) were in this category. If attention is paid only to the percentage of deaths, the fact that patients with thymomas fare less well is more obvious, since 58 per cent of them died as compared to deaths in 30 per cent of the entire group.

The percentage of good remissions among patients who had thymic tumors was eighteen; this is not significantly different from the 21 per cent good results for all patients comprising the study but it is in sharp contrast to the 50 per cent good results obtained in all patients without thymomas surviving thymectomy or the 53 per cent good results obtained in female patients who underwent surgical removal of non-neoplastic thymus glands. The higher death rate in the non-surgical patients with tumors is largely a reflection of the greater severity of myasthenic symptoms in this group. Most of the patients in this group were too ill to be considered for thymectomy and received roentgen therapy instead.

Although 26 per cent of the female patients who had tumors and who were treated surgically experienced satisfactory remissions, the groups were of such small size that this figure is not significantly different from the normal remission rate for female patients (20 per cent) and cannot be construed to indicate that thymectomy is of value in the treatment of thymoma. However, the fact that five of thirteen female patients with thymomas who survived thymectomy, an incidence of 39 per cent, attained desirable remissions may reflect a favorable trend. Actually, fourteen female patients survived surgery but one died of causes other than myasthenia gravis and consequently was eliminated from the study. A remission rate of 39 per cent, which is almost twice that in the female control group without tumors, cannot be interpreted as demonstrating that surgical removal of thymomas from female patients is definitely contraindicated. We believe that

more patients must be studied before definite conclusions are drawn regarding the value of removal of thymomas from female patients.

Some light is thrown on the question of malignancy of thymomas by study of this group of patients. The thymomas were considered inoperable in six of forty-one patients in whom the data have been analyzed; only biopsy was feasible because the tumor had invaded surrounding structures, including at times the pleura, lung, pericardium and great vessels at the base of the heart. The tumor in another patient was removed except for a nubbin of tissue adjacent to a major vessel. Subsequently the patient received roentgen therapy and died of myasthenia gravis; at necropsy, the tumor was found to have recurred. Another patient in whom the thymoma had been incompletely removed improved as far as the myasthenia gravis was concerned only to die as a result of recurrence of the tumor with obstruction of venous return to the heart. In seven patients the tumors, although they invaded the pleura, lung, pericardium or innominate vein, were considered to have been completely removed by resection of the invaded tissue. In three other patients the tumors were found to be adherent to but not definitely invasive of surrounding structures. Thus in this group of forty-one patients the tumors were locally invasive in fifteen patients and could not be completely removed in eight.

TABLE VIII

MYASTHENIA GRAVIS WITH THYMOMA

COMPARISON OF RESULTS IN FIFTY-FIVE SURGICAL AND NON-SURGICAL PATIENTS

(MAYO CLINIC SERIES, 1952)

Results	Female				Male				Total	
	Surgical		Non-surgical		Surgical		Non-surgical			
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent
+4 Complete remission	3	16 } 27	0	10 } 10	2	10 } 15	..	...	5	} 18
+3 Considerable improvement	2	11	1		1	5	1	17	5	
+2 Moderate improvement	3	16	..	.....	2	10	..	...	5	..
± No essential change	1*	5	1	10	5†	25	..	...	7	..
− Worse	0		0		1	5	..	...	1	
Died (surgical death)	5	26 } 52	0	} 80	...		..	...	5	
Died of myasthenia gravis	5	26	8	80	8‡	40 } 50	5	83	26	} 60
Died of invasion of great vessels by tumor	...	.....	..	.....	1§	5	..	...	1	
Total	19	100	10	100	20	100	6	100	55	..

* Died of cerebral disease (demyelinizing); myasthenia gravis in remission at time of thymectomy; no relapse at time of death.

Note: The tumors in eight of these fourteen patients († = one of the five; ‡ = six of the eight; § = one of one) could not be removed. Patients surviving surgery received roentgen therapy.

RISK OF THYMECTOMY IN MYASTHENIA GRAVIS

In deciding whether or not a patient who has myasthenia gravis is to undergo thymectomy the physician must weigh the risk to the patient of surgery against the chances of benefit from surgical intervention. In our series eight of 121 surgically treated patients died within three days of operation. Other deaths after thymectomy in this series have occurred at varying intervals but none sooner than several months postoperatively; consequently, they cannot be considered to have resulted from the operation. The over-all operative mortality rate has been 6.6 per cent.

As might be anticipated the mortality rate has been greater in patients who have thymomas, in

whom the average age is greater and the operation more difficult and prolonged. There have been five operative deaths in the fifty-one myasthenic patients undergoing surgical treatment for thymoma, a surgical mortality rate of 9.8 per cent.

The operative mortality rate in the Johns Hopkins University series was reported by Harvey[12] as three deaths in thirty-two patients; these three deaths occurred among the seven patients who had tumors. According to Schwab and Leland[18] the operative mortality rates in the Massachusetts General Hospital series were 7.5 per cent in fifty-three female patients and 24 per cent in twenty-five male patients. Both groups contained patients with and without tumors. Keynes[4] in 1946 reported eight operative deaths during his first fifty-one operations, two of which were among the six patients who had tumors. In 1949 Keynes[5] stated that ten operative deaths had occurred in 137 thymectomies in the absence of thymomas; five had occurred in the first eighteen operations and the remaining five in the next 119 operations. A higher mortality rate early in their experience has been the universal experience of all who have reported large series.

TABLE IX
MYASTHENIA GRAVIS
FOLLOW-UP RESULTS IN 312 PATIENTS
(MAYO CLINIC SERIES, 1952)

Results	Surgical				Non-surgical				Total		
	Tumor		No Tumor		Tumor		No Tumor				
	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent	Patients	Per cent	
+4 Complete remission	5	13	8	17	..	...	13	6	26	} 21	} 37
+3 Considerable improvement	3	8	14	29	2	13	21	10	40		
+2 Moderate improvement	5	13	9	19	..	...	34	16	48		
± No essential change	5	13	9	19	1	6	62	30	77	..	
− Worse	1	2	2	4	..	...	23	11	26	} 39	
−4 Dead*	20	51	6	12	13	81	56	27	95		
Total	39	100	48	100	16	100	209	100	312	..	

* *Analysis of deaths:* (1) Eight deaths occurred as a result of surgery; five of these patients had tumors and three did not. (2) Of the patients who died later of myasthenia gravis, thirteen surgical patients had tumors and two surgical patients did not; thirteen non-surgical patients had tumors and thirty-four non-surgical patients did not. (3) Other causes of death were noted in two surgical patients who had tumors, one surgical patient who had no tumor and twelve non-surgical patients who had no tumors. (4) The cause of death was unknown in ten non-surgical patients, none of whom had tumors.

Of the seventy myasthenic patients treated surgically for removal of a non-neoplastic thymus gland, three have died as a result of surgery, a surgical mortality rate of 4.3 per cent. These three operative deaths occurred among the first thirty-two patients operated upon for removal of non-neoplastic thymus glands; since November, 1946, no deaths have been attributable to surgical treatment in the non-neoplastic group, although thirty-eight such patients have undergone thymectomy during this period. Prior to 1952 only patients who had myasthenia gravis of moderate severity were selected for surgical treatment, whereas since 1952, when we became convinced of the value of thymectomy, the condition of not a few of the patients has been such as to make the risk of operation very high but the risk was accepted on the basis that thymectomy might prove to be lifesaving.

No useful purpose is served by comparison of the mortality rates in the various series, since details regarding the seriousness of myasthenia gravis in specific instances are lacking. Although

age, the general condition of the patient, the presence or absence of thymoma and the experience and ability of the surgical and medical teams are important factors in the evaluation of risk to the patient, experience has shown that when other factors are equal the really critical factor is almost always the severity of the myasthenia gravis. The risk of operation is directly related to the risk of respiratory failure. If the patient has experienced difficulty in breathing or in swallowing secretions in the throat, particularly if these symptoms are not eliminated by optimal amounts of neostigmine, the risk is significant. However, the performance of tracheotomy and the use of a mechanical respirator and aspirator, along with expert medical management of myasthenia gravis, usually allow the patient to survive any crisis that may be induced by operation. If the cough is so weak that the finer branches of the bronchial tree cannot be kept free from obstruction by secretions, particularly while optimal amounts of neostigmine are being administered, the risk of surgery to the patient is too great to accept and surgical intervention should be postponed.

Unfortunately medical management is difficult in severe degrees of myasthenia gravis. The chief difficulty lies, when crises arise, in deciding whether the patient has been receiving too much or too little neostigmine. In such instances we have found no substitute for tracheotomy, use of the respirator and mechanical forms of aspiration, the administration of oxygen by inhalation and close observation of the response to the administration or withholding of neostigmine.

On the other hand the risk of operation is not great provided the patient is young, can cough vigorously and has no significant difficulty in swallowing or breathing while receiving a relatively small amount of neostigmine. We would estimate the risk of death at operation in such patients as approximately 1 per cent. Of course it is well to anticipate and be prepared for trouble. Elective use of tracheotomy and the respirator is preferable to use of these aids as an emergency.

OBSERVATIONS

Use of Controls. At present, evidence overwhelmingly favors the conclusion that thymectomy is of value in the treatment of myasthenia gravis. A considerable amount of evidence is available regarding factors that have favorable or unfavorable influence on prognosis after thymectomy. Future studies promise further elucidation of the problem of selection of patients for thymectomy.

It is obvious that younger patients who have had myasthenia gravis for a short period are most likely to improve after thymectomy. On the other hand controlled studies indicate that these are the patients who are most likely to improve without thymectomy. Consequently the real value of thymectomy can be demonstrated only in control studies in which a comparison is made of the course in surgical and in non-surgical patients of the same age and in whom the duration of disease is the same. The number of patients in each group must be large enough to be statistically reliable. In this relatively early stage of our studies there is a temptation to draw conclusions from too few cases. Consequently we urge patience, persistence in study and reservation of opinion regarding the value of thymectomy in the categories that are now too small for use in the formulation of reliable opinions.

Two examples illustrate the point we are trying to make. (1) By combining the data from the Massachusetts General Hospital series and from our series, we have information regarding seventy-four female patients who had non-neoplastic thymus glands removed; these data can be compared with those from an even larger number of non-surgical controls. Complete recovery or considerable improvement occurred slightly more than twice as frequently among the surgically treated patients as among the controls. This appears to be the type of evidence deserving of confidence. (2) In our series we have follow-up data with regard to thirteen female patients who survived surgical removal of thymomas. As already stated, five patients (39 per cent) became well or greatly improved, a rate of remission which is almost twice that in female control patients who do not have thymomas. Although it is not improbable that these statistics reflect a trend favoring removal of thymomas in female patients, the question by no means can be considered settled. We believe it is well to withhold opinion under these circumstances while further data are collected and analyzed.

To summarize to this point, we can say that statistical evidence collected in controlled studies has established the value of removal of the non-neoplastic thymus gland in female patients who have myasthenia gravis. However,

the same studies do not at present indicate that thymectomy is of value as far as myasthenia gravis is concerned in males or in patients who have thymomas.

Relationship Between Myasthenia Gravis and the Thymus Gland. To clarify our attitude toward thymectomy in males, in patients who have thymomas or, for that matter, in any patient for whom the procedure has not yet been demonstrated to be of value, it is advisable to review briefly the nature of the relationship between myasthenia gravis and the thymus gland.

Weigert[29] in 1901 was the first to detect any such relationship when he described a malignant anterior mediastinal tumor at necropsy in a case of myasthenia gravis. This stimulated other pathologists to look for and describe abnormalities of the thymus gland in this disease. By 1939 the contributions of Bell,[30] Norris,[31,32] Miller,[33] and Blalock and co-workers[1] had established beyond reasonable doubt the fact that thymic tumors occurred too frequently in myasthenia gravis to be coincidental. Blalock's group were able to collect reports of fifty-three instances of abnormality of the thymus gland in approximately 110 cases studied at necropsy or surgical exploration. Of these abnormalities thirty-one were thymomas and twenty-two were instances of "persistence" or "hypertrophy."

Whatever reasons may have existed in the past for doubting the relationship between myasthenia gravis and the thymus gland have been abolished by studies of the thymus glands removed surgically during the era of frequent thymectomy. The work of Sloan,[34] Collins,[35] Bratton[36] and Castleman and Norris[37] has established that non-neoplastic thymus in myasthenia gravis is characterized not by variations in size and weight ("persistence" and "hypertrophy"), as had been claimed previously, but by histologic evidence of lymphoid hyperplasia with formation of germinal centers in the medulla. However, the frequency with which thymomas are now found in patients who have myasthenia gravis clinches the argument in favor of some sort of relationship. The study of thymomas published by Seybold and associates[38] is of signal importance and is recommended for anyone who desires to pursue the subject further.

The exact nature of the relationship between myasthenia gravis and the thymus is still under investigation. The superficially attractive hypothesis that the thymus elaborates a substance which induces the specific type of neuromuscular dysfunction recognized clinically as myasthenia gravis remains only a hypothesis. However, it has received recently its greatest support from the work of Wilson and co-workers[39] in England who found that extracts of thymus glands depress muscular contraction in various nerve-muscle preparations. The results of further controlled experiments by Wilson's group and the attempts of other investigators to corroborate their observations are eagerly awaited by clinicians. However, it appears evident that on clinical grounds alone the thymus gland does not always play a primary role in the production of myasthenia gravis since the disease at times progresses relentlessly to a fatal termination after removal of all detectable thymic tissue.

Thus clinical evidence does not favor the theory that the thymus stands in causal relationship to myasthenia gravis. A more attractive theory to us is that the basic disturbance in endocrine metabolism which probably gives rise to the specific type of muscular dysfunction recognized as myasthenia gravis also gives rise to the changes in the thymus gland characteristic of this disease. Removal of the thymus gland, when effective, may disturb the endocrine metabolic processes in such a way that they become more nearly normal with lessening of the severity of myasthenia gravis.

Thymectomy in Cases in Which Its Value Is Not Yet Demonstrated Statistically. Our reasoning in favor of continuing to do thymectomy in male patients, in patients with thymomas and occasionally even in older patients and those who have had myasthenia of long duration is based on two main concepts. First, as already indicated, a relationship exists between myasthenia gravis and the thymus gland but in all likelihood the abnormalities in the thymus do not cause myasthenia gravis. Removal of non-neoplastic thymus glands in female patients, at least younger ones, leads beyond reasonable doubt to remission of the symptoms of myasthenia gravis, probably as a result of altered metabolic processes. Second, although thymectomy has not yet been demonstrated by statistical methods to be effective in male patients who have myasthenia gravis and in patients who have thymomas, the symptoms of individual patients in these categories have gone into remission after thymectomy and there is no way of being certain that such improvement would have occurred without thymectomy. As an example, two men in our series, aged fifty-four and forty-five years,

respectively, became completely well within months after the removal of thymic tumors and had remained so for eleven and nine years, respectively, when last heard from. Another patient, a sixteen year old boy, was critically ill with myasthenia gravis and was becoming progressively worse at the time of thymectomy in July, 1954; he experienced considerable improvement within three months after surgery.

Consequently, until the time arrives when we can be certain that thymectomy will not help a specific patient or until something else of greater promise for induction of remissions is developed, we shall feel obligated to consider thymectomy for each patient. We propose to weigh the probable risk to the patient of surgical intervention against the probable chances of help, to give due consideration to all other factors that usually enter into decisions of this kind and, in each instance, to decide accordingly.

In patients who have thymomas, the locally malignant character of many of the tumors furnishes an additional indication for removal. For this reason and because of the possible beneficial effect on myasthenia gravis in female patients in particular, we intend to continue to advise thymectomy for patients who have thymic tumors, provided that either clinical or roentgenologic evidence of inoperability does not exist and the risk of surgical intervention does not appear to be excessive.

Retrospection and Prophecy. Keynes[7] has wondered why our 1949 study, published in 1950,[23,24] did not support the thesis that thymectomy was of value in the treatment of myasthenia gravis. Because our original series contained such a high incidence of patients with thymomas (33 per cent) among those who were treated surgically, that study did fail to reflect the true value of thymectomy, a fact that we were the first to recognize when doing our 1952 study. This abnormally high percentage of patients with tumors produced atypical results, and at the time there existed no good reason for suspecting the presence of such aberrant findings.

This experience makes us strongly suspect that any controlled series of cases containing a high percentage of tumors in which the results for male and female patients are not analyzed separately is unlikely to reflect the value of thymectomy when the series is relatively small. We predict that the Johns Hopkins University series eventually will support the use of thymectomy after more surgically treated patients, particularly female, without tumors are added. The better results of thymectomy compiled by Keynes may be a reflection of the highly selective composition of his surgical group. The great majority of his patients are less than forty years of age and it is in this age group that female patients with myasthenia gravis are known to predominate in the ratio of 2:1.

In 1949, as already mentioned, we did not appreciate the fact that inclusion of a high percentage of patients with thymomas could obscure the value of thymectomy. It was the reports of apparent success in the surgical treatment of three patients with thymomas that interested us in undertaking our studies in the first place. Nothing occurred during the early stages of our work to warn us that patients with tumors were to do poorly as a group. In 1947 we could report[20] that in our first fifteen surgically treated patients with thymomas, only one death, an operative one, had occurred and that seven of the eleven patients who were improved had done so to a pronounced degree. This relatively extensive experience with thymomas caused us to hesitate in the acceptance of warnings emanating from study of smaller series. The chronology of the various studies concerned also helps to explain some of the discrepancies between our earlier conclusions and those of Keynes[5] and of Viets.[16] Our 1949 study had been presented first, namely in June, 1949, at the meeting of the American Neurological Association. Keynes' study had been presented in July, 1949, as a lecture at the National Hospital for Nervous Diseases. Viets' study had been presented at the Fourth International Neurological Congress in September, 1949. However, ours was the last of these studies to appear in print.

In 1954 Keynes[7] said, "It is worth noting at this point that the Mayo Clinic had asserted a few years before that there was no convincing evidence that the thymus gland was in any way connected with myasthenia gravis." However, one of us (Eaton[40]) had proclaimed this relationship as early as 1942; the relationship between myasthenia gravis and the thymus has been discussed often by us in subsequent publications and its existence never has been denied.

SUMMARY AND CONCLUSIONS

1. The evidence found in our study combined with that in the literature makes it evident that thymectomy is of value in the treatment of female myasthenic patients who are less than

fifty years of age and who do not have thymomas. Too few patients who are alike except that they are more than fifty years of age have been studied to allow the formation of definitive conclusions. Younger female patients surviving thymectomy have better than a 50 per cent chance of attaining very satisfactory remissions, whereas without thymectomy they have a 20 to 25 per cent chance of attaining comparable results. Furthermore the chances of ultimate survival of patients in this group are substantially increased by removal of the thymus gland.

We approve of thymectomy in the case of female patients, particularly if they are young, in spite of the fact that surgery in severe degrees of myasthenia gravis is of great risk to the patient. It is probably not wise to advise thymectomy, even in female patients, if the disease has been present for many years (ten or more), particularly if it is stationary or improving.

2. The value of thymectomy in male patients has not been demonstrated conclusively up to the present time. We approve, tentatively, of offering thymectomy to male patients as of potential benefit unless the risk of surgery contraindicates the use of a procedure the worth of which is not yet conclusively established for such patients.

3. Thymectomy for removal of thymic tumors carries a relatively great risk to the patient and is not of proved value, although data in studies of the Mayo Clinic series suggest that it may prove to be of value in female patients. Perhaps it should be undertaken provided there is no clinical or roentgenologic evidence of inoperability and the risk of surgical intervention does not appear excessive. Otherwise a patient who is destined to do well as far as myasthenia gravis is concerned may die as a result of thymoma. Roentgen therapy is reserved for those patients who are unsuited for surgical treatment.

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Pregnancy in Myasthenia Gravis and Neonatal Myasthenia Gravis*

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SINCE my own experience with the problems of myasthenia gravis in pregnancy and during the neonatal period has been rather limited, I would like to introduce my presentation with some references to the literature.

Many authors have been impressed with the effect of pregnancy upon myasthenia gravis but their reports fail to serve as a reliable basis for predicting the effect in any particular instance. The occurrence of diametrically opposed reactions in relation to pregnancy adds another as yet unexplained peculiarity to the clinical course of myasthenia gravis. Perhaps most striking in this regard is the report of Laurent, who in 1931 described a patient who had had remissions during her first two pregnancies and severe exacerbations during five subsequent pregnancies, all of which terminated in abortions. In 1937 Kennedy and Moersch commented upon seven myasthenic patients who had twelve pregnancies. In five instances there was exacerbation during pregnancy with remission after termination in four; in the other seven instances there was no apparent effect during pregnancy but no mention was made of the puerperium. The most detailed study of the question of pregnancy in myasthenia gravis was reported by Viets, Schwab and Brazier in 1942. They concluded that in most cases the course of myasthenia gravis was "profoundly affected" by pregnancy, the usual course being characterized by a moderate relapse during the first trimester followed by partial or complete remission thereafter. In 1948 Harvey observed that pregnancy had a variable effect on myasthenia gravis but that exacerbations always occurred after the termination of pregnancy. In 1953 Fraser and Turner surveyed fourteen cases of myasthenia gravis in which pregnancy had occurred and concluded that there was no indication for termination of pregnancy although some tendency towards relapse was observed during the first trimester. They also reported that relapses in the early postpartum period occurred in half of their patients and apparently constituted the greatest period of danger.

It would appear that in the majority of instances pregnancy is associated with remissions during most if not all of the period of pregnancy. However in some instances, such as those reported by Wilson and Barr in 1945 and by Harris and Schneider in 1948, pregnancy may be associated with progressive exacerbation of myasthenia gravis, either with or without remission after the termination of pregnancy.

A preliminary and somewhat hasty survey of approximately 100 cases of myasthenia gravis that I have seen within the past fifteen years has disclosed four patients in whom six pregnancies have occurred. At first glance these cases would seem to illustrate the unpredictable reaction of myasthenia gravis during pregnancy. In the first case, in which the course of myasthenia gravis over an eighteen-year period had been relatively benign, the reaction during the first pregnancy was unchanged and during the second pregnancy showed only a slight exacerbation in the latter trimesters of pregnancy. In the second case the patient had had four illegitimate pregnancies and myasthenia gravis first developed three months after the termination of her third pregnancy. During her fourth pregnancy there was a rather marked remission which continued until three months after the termination of pregnancy when a slight exacerbation occurred. In the third case the course of illness during both the first and second pregnancies was characterized by initial exacerbation during the first trimester followed by a distinct remission which was maintained until two days after the termination of the first pregnancy and until eight days after the termina-

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tion of the second pregnancy. In the fourth case the course of myasthenia gravis showed a rather striking exacerbation during the last trimester with a partial remission in the postpartum period. The exacerbation in this case seemed to coincide with the probable development of hyperthyroidism. It is tempting to speculate in this case upon the possible detrimental effect of such thyroid dysfunction which has been also observed to occur in most patients in whom myasthenia gravis and hyperthyroidism coexist.

Interesting and possibly significant have been the relatively recent reports of a myasthenic syndrome affecting some infants whose mothers have myasthenia gravis. It would appear that this is an infrequent occurrence in view of the observation by Viets of only three examples in thirty-six myasthenia pregnancies. The affected infants have been considered to represent a neonatal form of myasthenia gravis in contrast to the congenital form which occurs in infants whose mothers do not have myasthenia gravis. The latter have a course of illness resembling that which is observed in children and adults; this includes an unpredictable duration, as well as exacerbation and remission. Two examples of the congenital type of myasthenia gravis were included in a series of eight children, all girls, who were reviewed in a paper which I presented at the 1954 meeting of the American Neurological Association. There have been only six other cases of congenital myasthenia gravis reported in the literature.

Twelve cases of the neonatal type of myasthenia gravis have been reported and, with the possible exception of one case, the mothers of these infants have been under treatment. TEPP was used in the treatment of one mother; the others received neostigmine. Thymectomies were performed upon three of the mothers. Myasthenia gravis was evident in these infants within a few hours to a few days after birth. The syndrome in the infants was characterized by symmetric weakness of the eyelids, face, and bulbar and skeletal musculature. The weakness was relieved by neostigmine whenever it was administered. The duration was reported to have varied from seven days to thirty-six days and in those infants who survived spontaneous and apparently permanent remission always occurred. No relapses have been reported as yet. Two of the affected infants died.

Although I have not had the opportunity of directly observing any examples of neonatal myasthenia gravis, it is probable that two of the four myasthenic mothers in my series had infants with neonatal myasthenia gravis. One of these mothers (the fourth case) had a pregnancy which terminated in the delivery of an infant who was cyanotic but started to breathe after the removal of thick mucus and application of artificial respiration. Crying was said to have been weak and death occurred one hour and thirty-five minutes after birth. Autopsy showed bronchial obstruction and cerebral congestion. There were no other abnormal findings and the thymus was apparently normal. Unfortunately this infant never received neostigmine. The other mother (the third case) has had two pregnancies and in each instance the infant apparently had difficulty in breathing and sucking. It is a noteworthy fact that the first pregnancy occurred before the mother received any treatment for myasthenia gravis. This infant's weakness, difficulty in breathing and difficulty in sucking subsided spontaneously within two or three weeks after birth. The second pregnancy terminated in May, 1954, with the delivery of an infant who had difficulty in sucking immediately after birth. This continued for six days and was relieved by the oral administration of neostigmine. In the literature the only indication of a similar occurrence of neonatal myasthenia gravis in siblings is noted in the report of Geddes and Kidd in 1951. They refer to a personal communication which described such infants following the third and fourth pregnancies of a myasthenic mother.

It has been suggested that neonatal myasthenia gravis is a result of placental transmission of a curare-like substance, the origin of which in the mother is obscure. Keynes has proposed the thymus as a source for such a substance in the mother but the reports of neonatal myasthenia gravis after thymectomy in the mother would be opposed to such a concept. If a curare-like substance is transmitted to the affected infant from the mother, it presumably is gradually excreted and destroyed and thus accounts for the apparently permanent remission if the infant survives. The question of suppression of normal neuromuscular humoral mechanisms in the affected infants because of intensive treatment in the mothers is opposed by the apparent occurrence of neonatal myasthenia gravis in infants whose mothers have not been treated.

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DISCUSSION

DR. KERMIT E. OSSERMAN: The influence of pregnancy on myasthenic symptoms and, conversely, the influence of myasthenia gravis upon gestation was studied by Drs. Nathan Kosovsky, Harold Spurt and the discusser in twenty-two myasthenic patients having a total of thirty-three pregnancies since 1940. Thirty-two per cent of the patients showed a definite remission during their pregnancy; 34 per cent showed no change in their myasthenic state, and 34 per cent showed a definite relapse associated with the pregnancy. All symptomatology patterns were usually established during the first trimester of the pregnancy and there was very little change during the second or third trimester, except in two cases. In those patients who had repeated pregnancies each pregnancy had its own influence on the myasthenia gravis and did not necessarily follow the pattern of the earlier pregnancy.

There were six therapeutic abortions and seven spontaneous miscarriages in this series. The occurrence of these spontaneous abortions bore no apparent relation to the degree of severity of the myasthenic symptoms which the patient presented. In the six cases treated by therapeutic abortions (these were the more seriously ill patients) interruption of the pregnancy did not change the severity of the myasthenic symptoms. However, following spontaneous abortion marked symptomatic improvement was observed.

Delivery and labor were normal, provided adequate cholinergic therapy was given. Cesarean section, as previously performed prior to 1937, is not required because of the myasthenia gravis but the decision to do this should be governed purely by obstetric considerations. The character of the labor was unaffected by the predelivery status of the patient. It is important to keep the patient at optimal neuromuscular function to the extent that this can be accomplished by cholinergic medication.

A three-month postpartum follow-up of most of the patients usually reflected the myasthenic status exhibited during the pregnancy. In those cases showing postpartum deterioration the added physical burden on the mother of the newborn may have been an added factor.

There are two classifications of myasthenia gravis in the newborn: (1) transient difficulty seen in children born of myasthenic mothers and (2) those rather rare cases of congenital disease in which no family history of myasthenia is obtainable. There were three occurrences of the acquired type in our series which resulted in sixteen live births. The physician should be on the watch for myasthenic symptoms in the newborn. This can be confirmed by performing either a tensilon® or neostigmine test. The baby can then be treated with cholinergic medication. The myasthenic symptoms usually abate completely in the first few weeks of life.

Endocrine Changes in Normal Pregnancy*

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PATIENTS suffering from myasthenia gravis frequently have a remission of the symptoms if pregnancy occurs, and it has been suggested that increased amounts of hormones elaborated during pregnancy may play a protective role in this disease.

Profound changes occur in the organs of reproduction during pregnancy and the body as a whole participates in many biochemical alterations in water, electrolyte, carbohydrate, fat and protein metabolism. As some of these effects are the result of increased elaboration of steroidal hormones, it seems pertinent to review some of the variations that occur in the secretion of hormones during normal pregnancy.

Gonadotrophins. In early pregnancy one of the most striking observations is the rapid rise in the concentration of chorionic gonadotrophin in blood and urine. (Fig. 1.) This substance is elaborated by the growing trophoblast for the purpose of maintaining the corpus luteum, causing it to continue to secrete progesterone and estrogens, thus preventing the collapse of the endometrial bed. Chorionic gonadotrophin appears in the urine very soon after implantation of the fertilized ovum and reaches a peak between the fiftieth and the seventieth day after the beginning of the last actually occurring menstrual period. The concentration of chorionic gonadotrophin in the blood follows a similar curve as that observed for urine.[2] The maximum level is maintained for only a short period and is followed by a rapid decrease in concentration. From approximately the one hundred tenth to one hundred twentieth day until the end of pregnancy this substance remains at a relatively low level. Following parturition it disappears from the urine within three to seven days.

Progesterone and Estrogen. The source of progesterone and estrogen in early pregnancy is the corpus luteum. This function is gradually taken over by the placenta. By the end of the second month the placenta in the human is secreting sufficient amounts of these steroids to maintain the pregnancy.

Because progesterone is rapidly metabolized in the body it is difficult to demonstrate its presence in either blood or tissue. Its chief metabolite is pregnanediol which is excreted in the urine as pregnanediol glucuronide.[3] Urinary excretion of pregnanediol in pregnancy is a reflection of the amount of progesterone elaborated. In very early pregnancy pregnanediol is excreted at the same level or slightly higher than that observed during the luteal phase of the menstrual cycle (4 to 10 mg./24 hours). Its concentration in the urine increases gradually during the first three months of pregnancy (Fig. 1), then more rapidly so that by the end of pregnancy from 40 to 130 mg. per twenty-four hours may be excreted. Following delivery of the placenta it disappears rapidly from the urine.[4]

Estrogen excretion in the urine during pregnancy follows a pattern similar to that of pregnanediol. (Fig. 1.) During the first three months it is present in the urine at levels slightly higher than those observed in the luteal phase, approximately 1 mg. per twenty-four hours. After the one hundredth day the concentration begins to increase and by the last month of pregnancy from 12 to 35 mg. per day may be excreted. The ratio of pregnanediol to estrogen in early pregnancy is roughly 10:1. In the last month it is 4:1. Thus the rate of estrogen elaboration increases more rapidly than that of progesterone. In Figure 1 is shown the interrelationship that exists between the excretion of various hormone metabolites in pregnancy.

Relaxin. Hisaw reported in 1926[5] that relaxation of the pubic symphysis in the guinea pig was under the control of a specific hormone, relaxin. Since that time it has been shown that mixtures of estradiol and progesterone are also able to induce pelvic relaxation. Relaxin can be detected in the blood of pregnant women.[6] Little is known regarding its mechanism of action.

* From the McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec.

Adrenal Cortex. There is much evidence that points toward an intimate relationship between the adrenal cortex and the reproductive system. The increase in the size of the adrenal glands observed in pregnant women[7] represents increased activity as judged by hormonal studies of blood and urine.

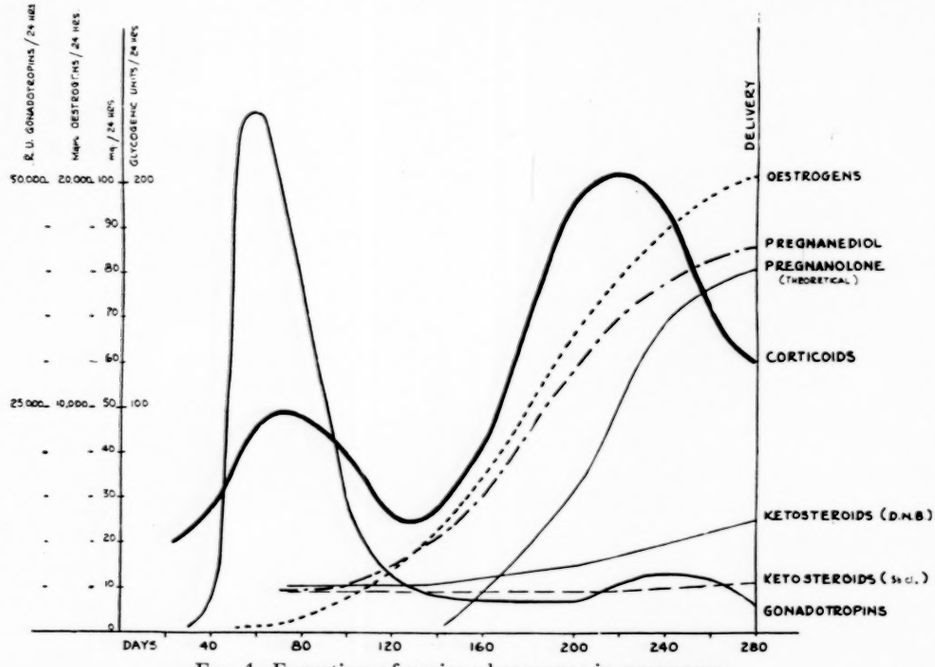

FIG. 1. Excretion of various hormones in pregnancy.

The adrenal gland elaborates a variety of steroidal hormones with different biologic activities. These include the glucocorticoids, the mineralocorticoids and a group of steroids which are metabolized and excreted as 17-ketosteroids. The glucocorticoids represent hormones such as hydrocortisone and cortisone which have a wide influence on tissue metabolism. Studies by Venning[8] show that in pregnancy there is an initial rise in excretion of glucocorticoids in the first trimester of pregnancy which usually returns to normal levels by the one hundredth to the two hundredth day. This is followed by a subsequent increase in the output of glucocorticoids which reaches a maximum excretion in the last trimester of pregnancy. This increased excretion of corticoids has been confirmed by chemical procedures.[9,10] Recent studies of the blood by Gemzell[11] showed that by the last trimester of pregnancy the blood 17-hydrocorticosteroids had increased fourfold. Venning et al.[12] reported that the average value for urinary corticoids causing sodium retention was slightly higher in the pregnant woman than in the non-pregnant woman. Little change was observed in the excretion of total 17-ketosteroids in pregnancy,[8] although it has been reported[13] that pregnant women excrete less androgen in the last month. Dobriner et al.,[14] reporting on isolation studies on the excretion of individual ketosteroids, found that the pregnant woman excretes ketosteroids which differ quantitatively and qualitatively from those found in the urine of non-pregnant women.

Thyroid Gland. In pregnancy the thyroid gland becomes more vascular and usually becomes hypertrophic, but it is not generally thought that this is accompanied by hyperfunction. An increase in the basal metabolic rate and the serum protein bound iodine[15] occurs. The significance of these observations is not clear.

Thymus Gland. Acute involution of the thymus as well as that of other lymphoid organs occurs during pregnancy. In human subjects maximal thymic atrophy is found within two months after onset of pregnancy.[16]

Selye[17] found that most hormonally active steroids will influence thymus involution in varying degrees; however, the estrogens and the adrenocortical hormones appear to be the most active in this respect. Of the adrenal corticoids the glucocorticoids have the greatest effect on thymic involution. These are the corticoids

which increase the muscle work capacity of adrenalectomized rats as shown by Ingle.[18] For some time it has been known that myasthenia gravis is frequently associated with some form of thymus lesion.

Metabolic Changes. One of the most characteristic biochemical alterations in late pregnancy is an increased retention of water. There is a progressive increase in plasma and total blood volume which becomes maximal in the ninth month. As a result of this dilution, the concentration of blood cells and haemoglobin is decreased.

It has been demonstrated that estrogens as well as adrenocortical hormones will cause water and sodium retention.[19] The period in which there is maximal water retention is associated with an increased elaboration of estrogens and adrenocorticoids. Estrogens also have a lipotrophic effect and possibly they are partly responsible for changes that occur in the total neutral fat, serum phospholipids and circulating cholesterol during pregnancy. These same hormones also affect calcium and phosphorus metabolism. Many pregnant women show a slight disturbance in carbohydrate metabolism which is due to a lowered renal threshold for glucose. Recent studies by Flynn et al.[20] on the occurrence of lactosuria and glycosuria in pregnant women showed that there is an increase in the incidence of glycosuria after the third month. It is well known that certain hormones of the adrenal cortex may affect carbohydrate metabolism and in pregnancy it is possible that the higher incidence of glycosuria may be associated with an increased adrenal function.

Various reports indicate that amelioration of symptoms in pregnant women with myasthenia gravis may occur at different periods. It is not unreasonable to suppose that the dramatic changes occurring in the endocrine glands and the consequent effects on metabolic processes in various tissues may have some influence on the course of the disease although it is not clear in what manner these remissions are brought about. In order to clarify this situation hormonal assays should be done on pregnant women with myasthenia gravis.

SUMMARY

The various changes that occur in hormone secretions during pregnancy have been reviewed. The first trimester is characterized by a rapid rise in levels of chorionic gonadotrophin in blood and urine. The function of the adrenal cortex is slightly increased at this time. In the last trimester, estrogens, progesterone (or its metabolite pregnanediol) and the adrenocortical hormones are at a high level in blood and urine. The relationship of the hormones to certain metabolic changes observed in pregnancy is discussed.

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Effect of Endocrine Glands on Normal Muscle Work*

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WHEN the gastrocnemius muscle of a normal anesthetized rat is made to contract by intermittent faradic stimulation, it can work vigorously for several days. By feeding the rats while they are working a high level of energy output can be sustained for ten days and longer; we have kept individual animals working continuously for as long as seventeen days. The rat loses its ability to work within a few hours after removal of either the adrenal glands or the pituitary gland but it is responsive to replacement therapy with the hormones of these organs. The ability of the experimental animal to work over long periods of time can be regarded as a criterion of vigor for it is a stressful procedure which will quickly reveal physiologic deficiencies not demonstrable under resting conditions.

METHODS

The details of the procedure have been described.[1] Male rats of the Sprague-Dawley strain were maintained on Archer Dog Pellets until they reached a weight of 200 ± 2 gm. The animals were anesthetized with phenobarbital sodium and cyclopal® sodium. The gastrocnemius muscle of the left hind leg is weighted with 100 gm. A nerve stimulator, model B (Upjohn), is used to deliver 5 pulses per second. The duration of each pulse is 20 milliseconds and the intensity is 20 ma. The distance the weight is lifted is recorded on automatic motion accumulators. Each recorder revolution represents approximately 400 gram-centimeters of work. The animals are enclosed in a cabinet with temperature constant at 28 ± 0.5°c. The test substances in aqueous solutions are injected into the jugular vein by means of a continuous infusion machine. When hormones are administered by intermittent injection into the tissues or a body cavity, the quantitative nature of the response may be affected by differences in the rates of absorption and in efficiency of utilization. The continuous intravenous injection of hormones simulates the manner in which the gland secretes its hormone into the blood more nearly than the procedures of intermittent injection. The fluid volume is 20 cc. per twenty-four hours per rat. By means of this apparatus (Fig. 1) twelve rats can be studied simultaneously.

EXPERIMENTS AND RESULTS

Studies on Adrenalectomized Rats. It has been known for a hundred years that clinical adrenal insufficiency is characterized by muscular asthenia.[2] In 1892 Albanese[3] demonstrated the muscular asthenia of adrenalectomized animals. In 1932 the author was encouraged by the studies on vigor by Dr. Roy Hoskins and his associates to test the effect of replacement therapy upon deficient work performance of the adrenalectomized rat. We observed that work performance fell below normal within a few hours following adrenalectomy and that this failure could be largely but not completely prevented by the injection of extracts of the adrenal cortex.[4] In 1934 the author joined the research group of Dr. E. C. Kendall who had supplied adrenal-cortical extracts for the initial research. When cortisone was isolated in 1935 by Mason et al.,[5] its biologic activity was immediately demonstrated in the work test, whereas it was considered to be inactive by other investigators. Subsequently 11-dehydrocorticosterone, corticosterone and hydrocortisone were isolated from adrenal cortical extracts and found to be active in this test.

When in the fall of 1938, 11-desoxycorticosterone was tested for its effect upon the work of adrenalectomized rats it was found to be

* From the Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois. This work was supported by grants from The American Cancer Society as recommended by the Committee on Growth and from the United States Public Health Service.

almost completely ineffective. This compound proved to be qualitatively deficient in its biologic effect upon work and upon organic metabolism just as the 11,17-oxygenated steroids were weak in their effect upon electrolyte balance. The capacity of an adrenal-cortical steroid to support work and to affect organic metabolism is dependent upon the presence of oxygen function at position 11 on the steroid molecule. The studies of Ingle and Kuizenga[6] demonstrated that the adrenal steroids can be ranked in the following descending order of activity when assayed by the muscle work test: hydrocortisone, cortisone, corticosterone, 11-dehydrocorticosterone and 11-desoxycorticosterone.

As shown by Ingle and Nezamis[7] the continuous intravenous injection of large amounts of beef adrenal extract can sustain normal work performance in adrenalectomized rats. Doses of 15 to 20 cc. per twenty-four hours per rat were required. Full replacement has never been achieved by the intermittent injection of adrenal hormones. None of the single steroid compounds are a full substitution for either the rat's adrenal cortices or for adrenal cortical extracts. When cortisone, hydrocortisone and corticosterone are mixed together in equal proportions the peak response is not superior to that elicited by optimal doses of the single compounds. Adrenal cortical extract is superior to pure compounds in supporting work.[8] (Fig. 2.) The recently isolated steroid, aldosterone, has not been tested for this property. It seems possible, even probable, that a mixture of aldosterone and hydrocortisone would fully support work performance just as does adrenal-cortical extract.

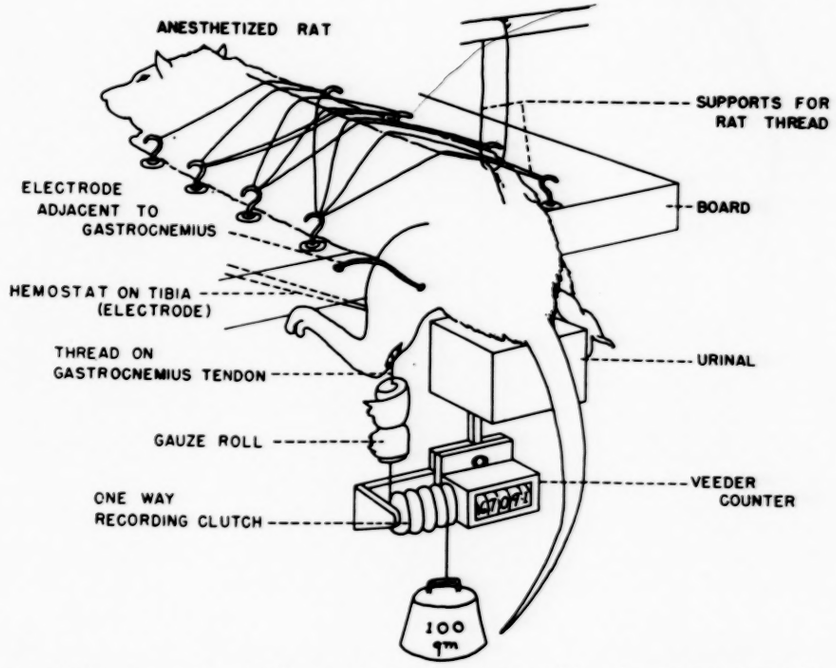

FIG. 1. Diagram of surgically anesthetized rat prepared for stimulation of muscle.

Treatment of the adrenalectomized "fa-

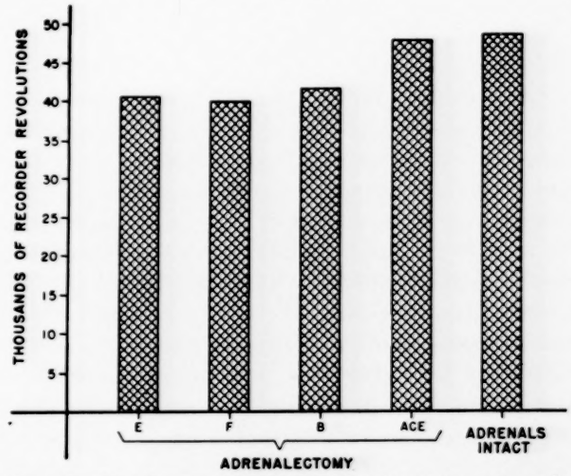

FIG. 2. Total amounts of work done by adrenalectomized-nephrectomized rats given optimal amounts of cortisone (E), hydrocortisone (F), corticosterone (B) or adrenal cortical extract (ACE) by continuous intravenous injection; average of fifteen rats per group.

tigued" rat with epinephrine will induce brief partial recovery but is incapable of prolonging the ability of the adrenalectomized rat to work. Adrenal demedullation causes the work capacity of the rat to decrease more rapidly than that of normal rats during the first ten hours, but thereafter the difference disappears and adrenal demedullated rats perform normal total amounts of work before death.[9]

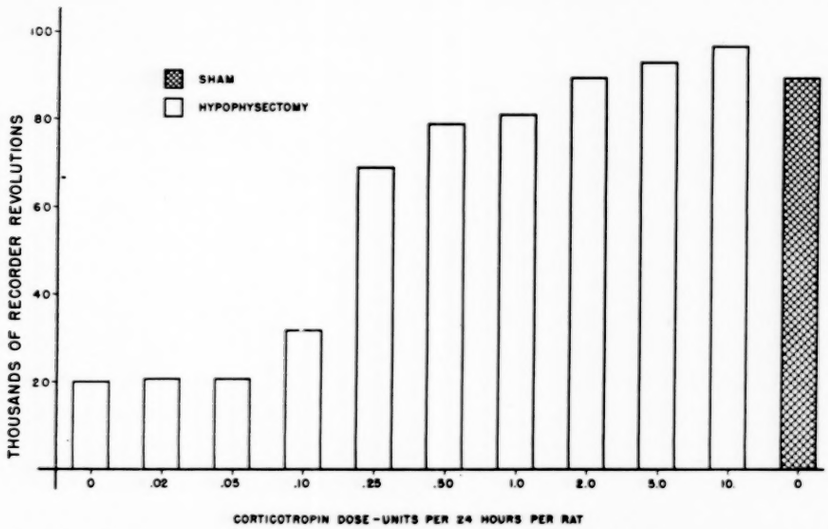

FIG. 3. Maintenance of normal work in hypophysectomized rats treated by continuous intravenous injection of corticotropin; average of fifteen or more rats per group.

Studies on Hypophysectomized Rats. The capacity of the rat to work in response to faradic stimulation is lost just as rapidly following hypophysectomy as following adrenalectomy. Indeed this failure is due in part to hypocorticalism which rapidly ensues after the source of the corticotropic hormone is removed.

Upon observing that continuous intravenous injection of commercial corticotropin sustained the ability of hypophysectomized rats to perform normal amounts of work (Fig. 3), it was supposed by us that the collapse of these animals in acute studies was due solely to hypocorticalism. To our surprise it was found[10] that adrenal-cortical extract only partially supports the work performance of either the hypophysectomized or the adrenalectomized-hypophysectomized rat, although it represented complete replacement therapy for the adrenalectomized rat. Moreover, the addition of corticotropin to adrenal-cortical extract supported normal work in the adrenalectomized-hypophysectomized rat. (Fig. 4.) At first we supposed that corticotropin itself has an important extra-adrenal effect, although we recognized that it might be contaminated with biologically important amounts of either known or unknown pituitary principles. In collaboration with Professor C. H. Li of the University of California it was shown that this "work" factor was separable from corticotropin. An optimal effect on work could be obtained with as little as 1 μg. per rat per day. Although this principle was present in all of a large number of preparations of corticotropin and other anterior lobe principles from a number of other laboratories, it could not be identified with any known anterior pituitary principle. After noting that a positive effect on work was correlated with an antidiuretic action, our attention turned to the hormones of the posterior lobe of the hypophysis. It is now established beyond reasonable doubt that vasopressin is the pituitary principle which supports the work performance of the hypophysectomized-adrenalectomized rat when added to adrenal cortical extract. This conclusion is supported by our tests of pure vasopressin* (Table 1). Further testing of the "work" fraction, which had been separated from corticotropin by Professor Li, showed that it contained approximately 2 international units of pressor activity per mg., an amount which could account for its positive effect on work. The mechanism of this effect is not known to us.

* Provided by Professor du Vigneaud of Cornell University Medical School, New York, New York.

Studies on the Diabetic Rat. We have a number of studies of the work performance of the diabetic rat, all supporting the conclusion that lack of insulin does not directly limit the ability of skeletal muscle to work or to utilize carbohydrate as a source of energy.[11]

Studies on the Effect of Castration. In studies done years ago when the conditions of the muscle work test were much less severe we failed to find any change from normal in the ability of either castrated males[12] or females[13] to do muscle work. These studies should be repeated.

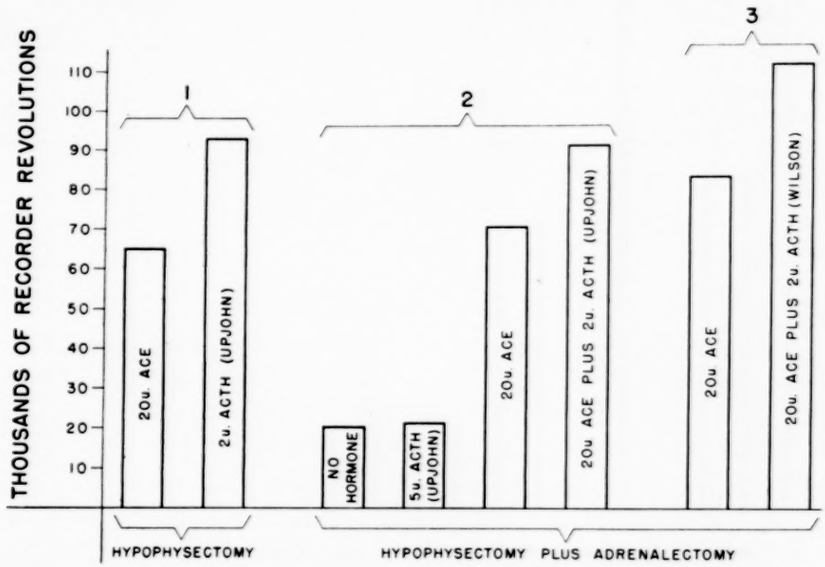

FIG. 4. The effect of some commercial preparations of corticotropin in the hypophysectomized rat and in the adrenalectomized-hypophysectomized rat with and without treatment with adrenal cortical extract; average for sixteen rats per group.

Studies on the Thyroid. Our studies on the relationship of thyroid function to vigor were of the cursory sort and have not been published. It was established to our satisfaction that the ability to sustain work output in the rat becomes subnormal during either severe hypothyroidism or hyperthyroidism. The changes from normal are much less dramatic than those caused by either adrenal cortical or hypophyseal insufficiency.

SUMMARY

When the gastrocnemius muscle of the anesthetized (phenobarbital sodium) rat is weighted with 100 gm. and stimulated to contract five times per second, it is capable of working for several days. The ability to continue work is rapidly lost following removal of the adrenal cortices or the pituitary gland. The ability of the adrenalectomized rat to work can

TABLE 1

EFFECT OF VASOPRESSIN UPON WORK PERFORMANCE OF ADRENALECTOMIZED-HYPOPHYSECTOMIZED RATS TREATED WITH ADRENAL CORTICAL EXTRACT

(Averages and Standard Errors)

Experimental Condition	Rats	Treatment	Total Average Recorder Revolutions
Sham hypophysectomy-adrenalectomy	16	Saline only	93665 ± 3293
Adrenalectomy-hypophysectomy	25	20 cc. adrenal cortical extract first 24 hr.; 10 cc. second 24 hr.	42130 ± 3623
Adrenalectomy-hypophysectomy	16	Adrenal cortical extract as above plus 0.02 units vasopressin per 24 hr.	93885 ± 2576

be brought to within normal limits by continuous intravenous injection of large doses of adrenal cortical extract. The 11-oxygenated steroids support near normal work performance and are much more potent than the 11-desoxy steroids. However, none of the pure steroids thus far tested will support normal work performance. Aldosterone has not been studied in the work test.

The work performance of the hypophysectomized rat can be brought to within normal limits by treatment with adrenal cortical extract plus vasopressin. Vasopressin is not effective in the absence of adrenal-cortical hormones.

Insulin is not directly essential for the support of muscle work. Other hormones have not been adequately studied, although it is known that vigor declines during either severe hypothyroidism or severe hyperthyroidism in the rat.

Acknowledgment. The rats used in these studies and the adrenal cortical extract were supplied through the kindness of Dr. M. H. Kuizenga of The Upjohn Company, Kalamazoo, Michigan.

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The Effect of Certain Endocrine Glands on Myasthenia Gravis*

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SINCE Thomas Willis' first description of myasthenia gravis in 1672, each of the glands of internal secretion have been thought by some investigators to be related either to the cause or the course of this disease. The pertinent literature on this subject up to the last decade has been reviewed by McEachern[1] and Goni.[2]

It may be stated at the outset that ten years later there is still no convincing evidence that myasthenia gravis is a specific type of endocrinopathy. However, it is possible to demonstrate that certain hormonal principles of the thyroid, adrenal and pituitary glands are able to modify the altered neuromuscular transmission seen in these patients. While the precise mechanisms of this action are still obscure, it is believed that this may well prove to be a fruitful avenue of approach to an understanding of the basic pathophysiology of myasthenia gravis.

In addition to the influence of pregnancy and menstruation on this disease (which is the subject of another presentation at this conference), perhaps the most interesting relationship is between the thyroid and myasthenia gravis. In discussing this we must first eliminate those patients with "chronic thyrotoxic myopathy" and "exophthalmic ophthalmoplegia"[3,4] who do not have true myasthenia gravis. This leaves a much smaller group—perhaps two dozen or so well documented cases—in which there is coincident hyperthyroidism and true myasthenia.[5,6,34] Most of the patients have been women between the ages of twenty to fifty-nine years who have shown characteristic improvement in their myasthenia with prostigmin.® In four instances in which the myasthenia preceded the thyrotoxicosis, the onset of the latter resulted in improvement in two of the myasthenics, while treatment of the hyperthyroidism made the myasthenia worse. This interesting "see-saw" relationship, originally commented on by McEachern,[5] has also been recently described by Maclean and Wilson.[6] Levitt[15] urges combined thyroidectomy and thymectomy in these cases and reports "good results" in six patients with thyrotoxicosis and myasthenia gravis so treated.

The cases described by Laurent[7] and Waldenström[8] and designated as "acute thyrotoxic bulbar palsy" or "acute thyrotoxic encephalomyopathy" are quite likely fulminant cases of myasthenia gravis associated with hyperthyroidism. That there is some "overlap" between these and the previously described cases is indicated by the sudden death (in respiratory failure) of three of twenty-one reported cases of "chronic" thyrotoxic myopathy[3,9,10] and the observation of McEachern and Ross[3] that prostigmin produced significant improvement in some but not all of these same cases. Finally, the interesting association between hypothyroidism and myotonia, the "mirror-image" of myasthenia gravis as far as neuromuscular transmission is concerned, may be mentioned.[11]

Three interesting laboratory observations are worth mentioning in connection with the thyroid gland. Tickner[12] is said to have found significant increase in serum cholinesterase levels in 50 per cent of thirty-five patients with thyrotoxicosis. Rawson[13] has reported that the thyrotropic factor (TSH) of the adenohypophysis is inactivated not only by iodides and thyroxine but also by thymus tissue. Finally, focal collections of lymphocytes ("lymphorrhages") are quite common in extraocular and somatic muscles in hyperthyroidism as well as in myasthenics.[14]

Nevertheless it is the rare patient with myasthenia gravis who will show any obvious clinical signs of thyrotoxicosis or any demonstrable alteration in the basal metabolism rate or serum cholesterol values. If there is a report of

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investigation of radioactive iodine uptake or serum protein-bound iodine studies in a series of myasthenia gravis patients, we are not familiar with it.

Coincidence of adrenal cortical insufficiency and myasthenia gravis is even rarer. In one such case personally reported[16] it was of interest that the patient failed to respond convincingly to prostigmin until fluid, carbohydrate and electrolyte values approached normal. Clinicians dealing with myasthenics are familiar with their poor tolerance to stress of almost any kind. Non-specific changes in the adrenal cortex have been described in myasthenics and a few enthusiastic reports of the efficacy of various adrenal extracts are on record. Sloan[17] found that the thymus gland in three of seven patients with Addison's disease showed changes similar to those seen in myasthenia gravis. Finally, Thevenard and Leger[18] have recently described improvement in four myasthenic patients subjected to denervation of both carotid sinuses, a procedure which in dogs is said to result in adrenal cortical hyperplasia.

Just how to integrate the diverse observations previously cited is a problem, but it is of considerable interest that thymic hyperplasia occurs in both Addison's disease and Graves's disease (as well as in castrates). Whether or not the thymus may provide the missing link, the overall situation currently is still very obscure and in critical need of reinvestigation by the powerful newer technics which the endocrinologist has developed in the past decade.

TABLE I

SUMMARY OF REPORTED RESULTS OF TREATMENT OF MYASTHENIA GRAVIS WITH CORTICOTROPIN (ACTH) AND CORTISONE

Authors	Total Treated	No. Improved	Marked Improvement	Slight to Moderate Improvement	No Change or Worse
			ACTH		
Schlezinger[22]	10	10	10	0	0
Torda and Wolff[23]	15	10	10	4	1; died third day
Wolfson[24]	4	3	2	1	1
Shapiro[25]	3	1	1	0	2; 1 died thirteenth day
Millikan and Eaton[26]	2	1	0	1	1
Soffer[27,28]	2	1	0	1	1
Stanbury[29]	1	1	1	..	.....
Sprague[30]	1	1	..	1	.....
Glaser[31]	1	1	1	..	.....
Grob and Harvey[32]	10	0	0	0	10
Ritter and Epstein[33]	1	0	0	0	1
			Cortisone		
Millikan and Eaton[26]	3	1	0	1	2
Grob and Harvey[32]	3	0	0	0	3
Schlezinger[22]	1	1	0	1	0

The third chapter in the "endocrine story" of myasthenia gravis became possible in 1943 with the isolation of the adrenocorticotropic factor (ACTH) of the anterior lobe of the pituitary in "pure" form in appreciable quantities by two groups of workers.[19,20]

Corticotropin was first used in the treatment of myasthenia gravis by Torda and Wolff[21] at the New York Hospital in 1944. They gave the following rationale for its use in these cases: (1) removal of the pituitary gland in rats induces changes in the electromyogram which closely resemble those seen in patients with myasthenia gravis; (2) the pituitary glands of several patients dying of this disease have shown an eosinophilic colloid material "suggesting altered function"; (3) administration of corticotropin (ACTH) causes shrinkage of the thymus and enhances synthesis of acetylcholine.

Corticotropin first became available commercially in 1948. Since then, a dozen references have been found concerning the use of it

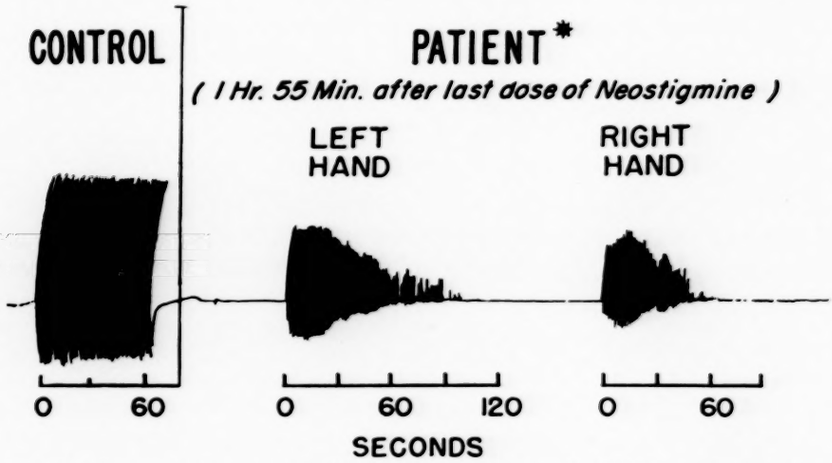

FIG. 1. Myogram: first day of ACTH therapy (unpurified) (40 mg. intramuscularly every six hours for eight days). Technic: all records taken at same interval following neostigmine. Recordings on Bell kymograph; speed 2.3 cm./min. Patient asked to squeeze bulb maximally once a second.

*Patient confined to bed, receiving 3 mg. neostigmine (H) every two hours.

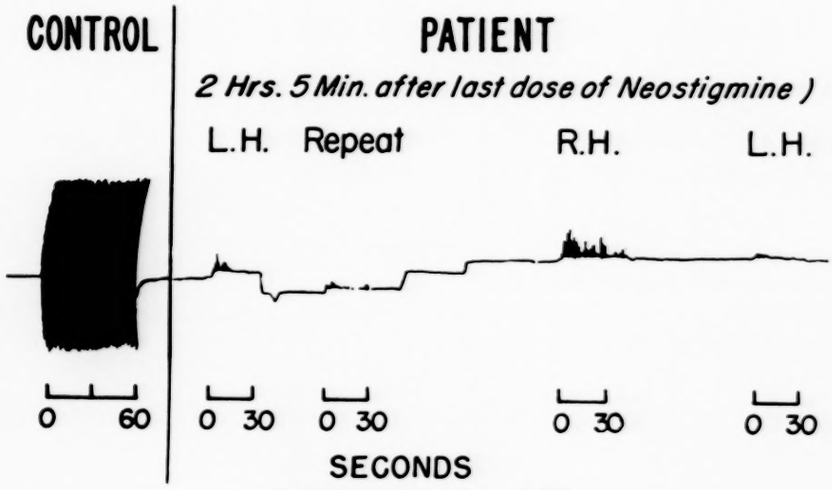

FIG. 2. Three days after ACTH.

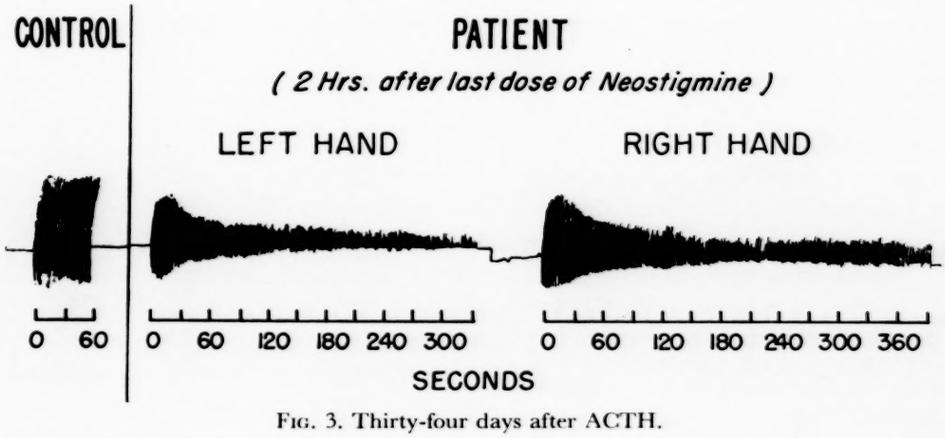

FIG. 3. Thirty-four days after ACTH.

(and cortisone) in sixty patients with myasthenia gravis. The reports are summarized in Table 1 and a typical response to corticotropin in a severe myasthenic is shown in Figures 1 to 4.

CONCLUSIONS

On the basis of a study of these reports and our personal experiences with the use of various

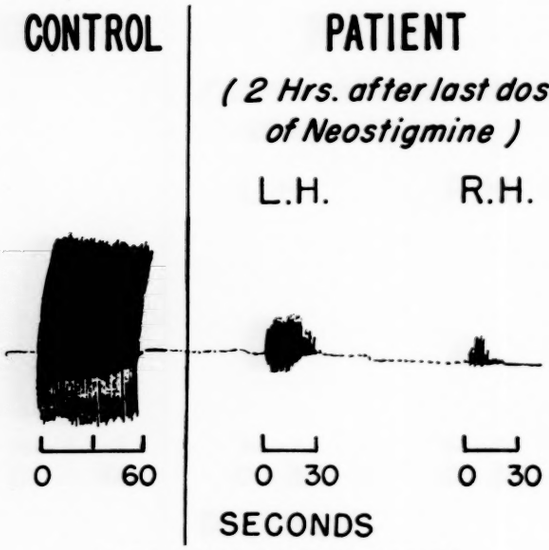

FIG. 4. Fifty-four days after ACTH.

pituitary fractions over the past three years, the following general conclusions seem justified: (1) Neither cortisone nor corticotropin is a panacea for myasthenia gravis; (2) the results with cortisone are disappointing and probably warrant little further study; (3) the results with corticotropin indicate, as a general pattern, initial worsening, improvement varying from slight to striking, beginning usually at the termination of therapy and lasting usually a matter of weeks and (4) despite the two deaths occurring during corticotropin administration to gravely ill patients, the results (over 50 per cent "markedly improved") cannot be disregarded in the light of our currently limited therapy.

The basic question, then, to be answered by future, better controlled investigations is not "does corticotropin help some myasthenics," but "how does it work?" and "could the pituitary-thyroid-adrenal axes be primarily responsible or contributory and not the thymus?" A coordinated "team-approach" combining the disciplines of the electrophysiologist, neurologist and endocrinologist will have to be achieved if further progress is to be made in this field.

Meanwhile, it is our personal belief that corticotropin still has a definite if distinctly limited contribution to make in treating myasthenic patients. We would define this as follows: (1) not justified in the mildly or critically ill patient; (2) best used in moderately severe myasthenic patients especially those with a radiologically enlarged thymus. In these patients the clinical and x-ray response may furnish a useful prognostic guide in the event of anticipated surgery (thymectomy); (3) treatment must be in a hospital equipped with adequate facilities for special tests and the care of respiratory emergencies.

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WIN 8077 in the Treatment of Sixty Myasthenia Gravis Patients*

A Twelve-month Report

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SINCE the successful introduction of oral tablets of neostigmine bromide in the treatment of patients with myasthenia gravis there has been a continued search for a better substance. One disadvantage of neostigmine is its short action; patients with severe

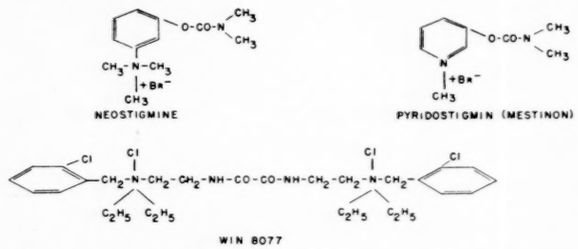

FIG. 1. Structural formulas of neostigmine, mestinon and WIN 8077. (From *J. A. M. A.*, 158: 625, 1955.)

cases have to take the medication every hour or so throughout a twenty-four-hour period. Even when this is achieved there may be a tendency for the drug to lose its effectiveness toward the end of this short interval. Furthermore, some patients are sensitive to this drug so that they have muscarinic effects of the cholinergic substance when the dose is increased sufficiently high to neutralize the symptoms of myasthenia gravis.

The use of belladonna preparations with each neostigmine dose, which would neutralize these unpleasant side effects, carries with it a certain risk. Dangerous toxic side effects can result when the warning signs of intestinal stimulation are thus hidden.

In November, 1953, an entirely new preparation[1,2] was made available for experimental trial by the Sterling-Winthrop Foundation. This preparation, called WIN 8077,† is N,N′bis(2-diethylaminoethyl) oxamide bis-2-chlorobenzyl-chloride, whose structural formula can be compared with neostigmine and pyridostigmin. (Fig. 1.) This compound is a white crystalline powder with a molecular weight of 608 and a melting point of 184.3°C. It is very soluble in water and can be sterilized by heat without alteration of its properties. It has a strong anticurare effect which is ten times that of tensilon and two to three times more than that of neostigmine and is of longer duration. Its anticholinesterase activity is nearly the same as neostigmine. No chronic toxic effects to the kidneys, liver or blood-forming organs were found in animal toxicity studies. Overdosage produced the same effects in animals as neostigmine, namely, vomiting, diarrhea and salivation. A preliminary report of its trial in myasthenia has already been made.[3]

TECHNIC OF EVALUATION

Like any new compound which has no known dosage levels in patients, the preliminary testing involved the use of minute amounts of the substance. Intravenously, in doses under 50 μg., no demonstrable effect on a patient with known myasthenia gravis was noted. It was not until the intravenous level of 250 μg. was reached that definite antimyasthenic effect was obtained. With this information oral trial of the medication was started in a number of patients in order to determine an effective dosage level. The first

† This drug is now being processed for release by the Food and Drug Administration (January, 1956) under the name of mysuran®.

* From the Neurology Service and Myasthenia Gravis Clinic of the Massachusetts General Hospital, Boston, Mass. This investigation was supported by a grant-in-aid from Winthrop-Stearns, Inc., New York, New York, who kindly supplied the material used in this investigation.

form of the preparation WIN 8077 was a syrup. This allowed a gradual increment of dosage without the necessity of working with fractions of tablets. We found that some patients had a definite response with 5 mg. per dose while others required as much as 50 mg. With this information available we recommended the clinical evaluation of 10 mg. scored tablets and a syrup containing 3 mg./cc. Patients who were adequately adjusted to neostigmine were transferred to the new preparation tentatively substituting 7½ mg. of WIN 8077 for 15 mg. of neostigmine bromide. The substitution was gradual at first so that patients continued to take both drugs during the period of adjustment. We found that this technic was not desirable since the two drugs seemed to have different lengths of action and there was a tendency for toxic side effects to occur too readily with this method. Subsequent changeovers involved abrupt switching of the patient to the new compound and cessation of the administration of neostigmine. This method proved more satisfactory and did not involve the patients in undermedication for more than a day or two.

Criteria for Effectiveness of the New Drug. A five-point numerical evaluation with 4, 3, 2, 1, 0 was used for scoring the value of this new compound. A top score of 4 goes to a drug which is objectively effective against symptoms in a clear and certain manner when taken by a patient in the absence of any other drugs. However, if the drug produces side effects requiring the presence of another drug to neutralize them the clinical value would be only 3. In this category it is assumed that the beneficial effect of the second drug is only associated with neutralization of the side effects of the first, and has no specific properties against the symptoms of the disease. A score of 3 also requires unequivocable and objective evidence of effectiveness. Category 2 applies to drugs which objectively assist another drug to improve the patient. Category 1 contains drugs that have a doubtful effect when linked with active compounds or a largely subjective one reported by the patient. Category 0 contains drugs with absence of both subjective and objective data as evidence of effectiveness.

In the study of WIN 8077 a sufficient number of patients taking only this preparation with definite objective improvement place this substance in Category 4.

Side Effects. Side effects from WIN 8077 are generally no different from those of neostigmine and other cholinergic drugs used in the treatment of myasthenia gravis. Patients who are sensitive or receive too much of the drug complain of excessive salivation, cramps, intestinal peristalsis, diarrhea and frequency of urination. These muscarinic effects are less likely to occur than with neostigmine bromide. The excessive salivation with WIN 8077 occurs before cramps and diarrhea, suggesting that the drug is more specifically powerful as a secretory stimulant. Dangerous side effects associated with the beginning of cholinergic block occur with this drug sometimes before cramps and diarrhea are evident. These are slowing of the pulse, myosis of the pupils, fasciculatory twitchings of the muscles of the tongue, shoulder or arms, and gradual onset of weakness of all muscles.

Precautions in Administration. It has been found that this drug has roughly twice the effectiveness per milligram of neostigmine in reducing the symptoms of myasthenia gravis, and approximately twice the duration. This prolonged effect may persist for as long as six hours. It is for this reason that the use of this preparation requires more care than neostigmine in the avoidance of side effects. It is better to undermedicate the patients for a number of days in switching them over from neostigmine than to risk overstimulation which may be dangerous. When given parenterally, either intravenously or intramuscularly, WIN 8077 exerts the same prolonged effect as by mouth. Therefore, particular caution must be used with an injection of this preparation since the effect may last several hours, making such overdosage doubly hazardous. The author recommends that the drug not be used parenterally, without special precautions, until more data can be obtained. For further evidence of overdosage the cautious use of intravenous tensilon,® 2 mg., may be tried. A transient increase in symptoms confirms cholinergic overdosage.

In any case of overdosage with WIN 8077 the prompt use of intravenous atropine sulfate, 1 mg., temporary cessation of all cholinergic therapy, and use of a respirator if necessary may be life-saving measures.

CASE REPORTS

CASE 1. A twenty-seven year old man with myasthenia gravis of two years' duration, involving ptosis and dysphagia, was successfully adjusted on neostigmine bromide (eight tablets per day). After a number of weeks an itching,

papular rash developed which bothered him considerably. This rash disappeared when the neostigmine bromide was removed. The patient had previously been sensitive to bromides by mouth. The physician in charge was at a loss to know any way in which the extreme sensitivity to bromine could be overcome in order that the patient's myasthenia gravis could be brought under control. WIN 8077 was substituted, five 10 mg. tablets per day, and excellent neutralization of his myasthenia resulted. No skin reaction occurred and the patient has been receiving WIN 8077 for the past six months. This is an unusual sensitivity to the bromine in neostigmine bromide but it does illustrate one of the advantages of WIN 8077.

CASE II. A forty-three year old woman with myasthenia gravis for five years, involving ptosis and diplopia as well as general weakness, was extremely sensitive to adequate doses of neostigmine bromide. She had diarrhea and cramps if the dosage was at a therapeutically effective level. Mestinon bromide was substituted for neostigmine bromide and the gastrointestinal effects were not present, but the antimyasthenia effect of this preparation in this particular patient was not as satisfactory as with neostigmine. She still complained of considerable weakness and diplopia when taking twelve 60 mg. tablets of mestinon bromide per day. She was changed over to WIN 8077 and immediately reported the same benefit she had had with neostigmine but without disagreeable gastrointestinal side effects. She was regulated on eight 10 mg. tablets of WIN 8077 per day without the appearance of any diplopia during the twenty-four-hour period. She has been maintained on this preparation for twelve months without any need of altering the dosage or increasing the amount per day.

CASE III. A forty-seven year old woman in two months developed bilateral ptosis, diplopia and some general weakness. An ophthalmologist diagnosed myasthenia by the prostigmin test which produced quite a severe gastrointestinal upset. A tensilon test confirmed the diagnosis without any side reactions. The patient, because of her reaction to neostigmine, was started on WIN 8077 in liquid form, 3 cc. of the elixir four times a day, with excellent control of her ocular symptoms. When she tried the 10 mg. oral tablets of WIN 8077 she had cramps, nausea and anorexia. With the elixir for a second time, 9 mg. per dose (3 cc.), there were no side effects. These recurred when tablets were tried once more. She is now comfortably regulated with the liquid. It is difficult to be sure whether the 1 mg. difference in the dose or the physical form of the preparation is responsible for this marked difference in tolerance. Some patients do much better on liquid medication and this case emphasizes the need of such a form for its administration.

CONCLUSIONS

WIN 8077 is a new effective substance to use in the treatment of myasthenia gravis. It has been employed successfully in forty of a total of sixty patients during the past 12 months. The side effects are similar to neostigmine and mestinon but are less likely to produce gastrointestinal disturbances when the therapeutic dosage is reached. It has a longer action than neostigmine and therefore it must be used with caution. Its parenteral use is not advised at this time.

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Progress Report on Mestinon Bromide (Pyridostigmine Bromide)*

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NEOSTIGMINE has withstood the test of time and has long been the undisputed drug of choice for the treatment of myasthenia gravis.[1] It has been effective in the treatment of the great majority of patients with this disease, especially those with the milder types of myasthenia gravis for whom relatively small doses of neostigmine are necessary.

The two chief disadvantages of neostigmine are its undesirable side effects such as gastrointestinal distress and its relatively brief duration of action. A third and most important disadvantage is that resistance to neostigmine may occur in severe myasthenia, so that even with increased dosages one cannot completely reverse the marked weakness of the patient.

There are many new cholinergic drugs available for research in myasthenia gravis today. One of these is mestinon® bromide (pyridostigmine bromide),† which is a dimethyl carbamate of 3-hydroxy-1-methyl pyridinium bromide. Preliminary results obtained with mestinon in the Mount Sinai Hospital Myasthenia Gravis Clinic were recently reported.[2] At the present time there are 283 patients reported in the American literature[3] and fifty-six in the European literature[4] who have received this drug. There are approximately 750 to 1,000 patients throughout this country who are now receiving mestinon therapy.[5]

In evaluating any new drug therapy for myasthenia gravis, we believe the following criteria must be considered: (1) What is the duration of action of the drug? (2) What relief of myasthenic symptoms does it offer? (3) Is the drug non-toxic? (4) Can the drug be helpful when the patient is resistant to neostigmine?

† The mestinon bromide used in this study was supplied by Dr. Thomas C. Fleming of Hoffmann-La Roche, Inc., Nutley, N. J.

METHODS

In the past year and a half eighty-one patients from the Myasthenia Gravis Clinic of the Mount Sinai Hospital have been treated with mestinon. The age and sex groups of these patients are listed in Table 1. The youngest age was thirty-eight weeks and the oldest eighty-three years.

TABLE 1
AGE AND SEX DISTRIBUTION OF EIGHTY-ONE PATIENTS

Age (yr.)	Male (25)	Female (56)
0–10	..	4
11–20	2	3
21–30	..	10
31–40	4	24
41–50	6	9
51–60	8	4
61–70	2	2
71–80	2	..
81–90	1	..

The severity of myasthenia gravis in a patient was estimated on the basis of cholinergic medication needed, but was classified also according to the economic usefulness of the patient to the community. Severe cases were considered to be those patients so affected by their myasthenia gravis that, despite therapy, they were bedridden or so weakened that they were unable to work; moderate cases were those patients who took relatively large doses of cholinergic medication in order to function and required frequent periods of rest during the day; and mild cases were those patients who maintained their economic place in society, were fully employable, attended school, or performed housework regardless of dosage of cholinergic medication, usually comparatively small. Our group of eighty-one patients before treatment with

* From the Myasthenia Gravis Clinic and the Department of Medicine, The Mount Sinai Hospital, New York, N. Y.

mestinon fell into the following groups: severe, twenty-four; moderate, twenty-one; mild, thirty-six.

Patients were admitted to the hospital for transfer from neostigmine to mestinon and the technic of serial tensilon® testing[6] was used to secure a quantitative comparison between neostigmine and mestinon in the same subject and to determine any difference in the duration of action of the two drugs. Subsequently, patients were given mestinon in the Clinic, merely substituting a 60 mg. tablet for each 15 mg. neostigmine tablet. More recently we have started new patients directly on mestinon therapy in the Clinic.

All of our patients have been studied by routine x-rays of the chest for thymic enlargement, electrocardiography, radioactive iodine and blood iodine studies, frequent blood counts including platelet counts and, in many cases, bone marrow examinations. In addition, routine urinalysis and physical examinations are performed at each clinic session. The effect of mestinon on the hematopoietic system was thoroughly investigated. We have found no evidence of any alteration to date.

The results of serial tensilon testing showed that the duration of action of mestinon is only slightly more protracted than that of neostigmine (approximately one-half hour longer). Mestinon cannot be considered a longer-acting cholinergic medication. The frequency of its administration during the day shows no appreciable advantage over neostigmine.

However, many patients, particularly those in the group with severe symptoms, were most gratified in that they could arise in the morning without severe myasthenic symptoms. This was most important to those patients receiving neostigmine who could not swallow their first morning dose because of dysphagia and therefore had to resort to an injection of neostigmine methyl sulfate to start the day. This nocturnal relief with mestinon has become increasingly apparent with further transference of patients so that now we do not prescribe mestinon during sleeping hours except on extremely rare occasions.

Mestinon is more effective than neostigmine in the relief of the small muscles innervated by the cranial nerves. Particular relief was noted in the muscles involved in ptosis, diplopia and dysarthria.

A striking advantage of mestinon over neostigmine is in the marked reduction in the incidence of muscarinic side effects such as epigastric distress, abdominal cramps and diarrhea, and in the relief of nicotinic side reactions such as skeletal muscle cramps. This reduction in side reactions was so pronounced that atropine could be discontinued in 75 per cent of the sixty-eight patients receiving atropine with neostigmine.

TABLE II

RESULTS BASED ON PRODUCTIVE CAPACITY IN EIGHTY-ONE PATIENTS

Drug Administered	Severe	Moderate	Mild
Original medication:			
Neostigmine	24	20	33
None	0	1	3
Final medication:			
Mestinon	8*	12	53
Neo-mestinon combination	0	1	2
WIN-mestinon combination	2	2	0
WIN 8077	...	..	1

* Including five deaths.

Schwab has commented on the possibility that in the absence of the early warning symptoms of overdosage special attention would be necessary to avoid cholinergic effects. We found no difficulty on this account because the usual muscarinic side reactions occurred as soon as overdosage of mestinon was reached. In fact, it is easier to avoid overdosage with mestinon than with neostigmine because of the wider range between therapeutic and toxic effect. Of course, in any question of doubt resorting to a tensilon test quickly resolves the problem.[7] (Table II.)

Upon transfer to mestinon the twenty-four patients who were considered severely incapacitated while treated with neostigmine showed such an improvement that fourteen of them could be reclassified in the moderate or mild group. Of twenty-one patients who were originally in the moderate group, six were so improved as to fall into the mild group. Thus, 45 per cent were improved enough to overcome their economic liability to their families and to the community.

Thirty-one patients had excellent results in that their myasthenia was improved. Twenty patients had good results in that improvement in myasthenic symptoms occurred but was not as notable as in the excellent group. Eight patients found no difference in the action of neostigmine and mestinon, and five patients preferred neostigmine to mestinon. In addition, there were four remissions in the group of eighty-one patients. (Table III.)

Some patients in the severe group, when first

transferred to mestinon, did not seem to be improved in any noticeable degree. However, with the continued use of mestinon over many months, there was a gradual but marked improvement in these patients. Of course, in a disease characterized by remissions one is reluctant to attribute the improvement to a drug. Nevertheless, the type of improvement seen was not that typically observed in a remission.

TABLE III
COMPARATIVE RESULTS OF MESTINON TO NEOSTIGMINE BASED ON IMPROVEMENT IN MYASTHENIC SYMPTOMS

Results	Mestinon	Neo-Mestinon Combination	WIN 8077-Mestinon Combination	WIN 8077
Excellent	31	2	1	1
Good	20	1	2	..
No difference	8	..	..	..
Poor	5	..	1	..
Deaths	6*	..	..	..
Remissions	4	..	..	..

* See text regarding one death which appears twice in table.

Attempts were made to improve the effectiveness of mestinon in patients in whom an excellent result was not obtained. Ephedrine sulfate, 3/8 to 3/4 gr. (25 to 50 mg.), was added to the maintenance dose of mestinon without any apparent improvement.

A number of patients were given a mixture of neostigmine and mestinon on a one-to-one or one-to-two basis in an attempt to increase limb strength and to give the "lift" which they desired. We were able to achieve two excellent results and one good one using this combination.

In view of a marked increase in limb strength noted with WIN 8077,* we tried this drug alone and in combination with mestinon in twelve patients. One excellent result was obtained with WIN 8077 alone and one excellent, two good and one poor result with combined WIN 8077 and mestinon therapy. We have no evidence of any synergistic action of neostigmine and mestinon, and apparently none between WIN 8077 and mestinon.

To the present time we have not found mestinon, WIN 8077 or any mixture of drugs effective when a patient is resistant to neostigmine.

There were six deaths in myasthenic crisis in the severe group; one of these occurred when a patient was admitted to another hospital and was not recognized as a myasthenic. Cholinergic medication therefore was not given.

Our mortality rate is 6.3 per cent. There have been instances when acute myasthenic crisis was relieved by mestinon, due to the marked decrease in side reactions which permitted the use of larger doses of mestinon without severe and uncomfortable muscarinic distress.

COMMENTS

We have tried to answer the four criteria proposed in evaluating any new drug therapy for myasthenia gravis: (1) As to the *duration of action* of mestinon, we find it only slightly longer-acting during the diurnal hours and therefore of no appreciable advantage over neostigmine. However, the effect during the nocturnal hours is much more prolonged with mestinon, permitting the patient a full night's sleep. (2) Mestinon gives marked *relief from myasthenic symptoms*, particularly those of the bulbar type. Results were excellent or good in 72 per cent of our series. Forty-five per cent of our patients in the severe and moderate groups were so improved as to overcome their economic liability. (3) Mestinon is definitely *less toxic* than neostigmine; this is one of its most striking advantages. (4) Through its lack of toxicity mestinon can be *helpful on occasions when the patient is resistant to neostigmine*. However, as yet we have no drug or combination of drugs which is effective when a patient in a myasthenic crisis is resistant to neostigmine.

Although mestinon is not the long-acting drug that we hoped to find, it is a valuable step forward in the search for the ideal drug in the treatment of myasthenia gravis and for the reasons already given, a superior drug to neostigmine. We still need a drug that is long-acting for the entire twenty-four hours of the day and one in which resistance does not develop. This search will be continued.

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* Mysuran.®

Management of Myasthenic and Cholinergic Crises

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The term crisis, as applied to myasthenia gravis, usually implies failure of muscles concerned with respiration and/or deglutition to such an extent that neither function can be maintained without mechanical aids. A true myasthenic crisis may occur in an untreated or insufficiently treated patient with severe myasthenia. Apparent resistance to antimyasthenic drugs develops in an occasional patient, resulting in crisis.

Only recently have we recognized that cholinergic drugs in excess can bring about a crisis.[1] This cholinergic crisis may be difficult to distinguish from true myasthenic crisis. It is possible that many patients previously thought to be in crisis due to drug resistance were actually in cholinergic crisis.

Grob et al.[2] were the first to report the syndrome of cholinergic crisis in non-myasthenics exposed to anticholinesterase insectides, and later, to "nerve gas."[3] He classified the symptoms as muscarinic, nicotinic and central nervous system in type. Muscarinic symptoms and signs, in order of severity, include anorexia, salivation, nausea, sweating, lacrimation, blurred vision, miosis, abdominal cramping, diarrhea, tenesmus, urinary frequency, excess bronchial secretion, dyspnea, substernal pressure, pulmonary edema and incontinence of bowel and bladder. Nicotinic symptoms and signs include muscle fasciculations, "thick tongue" with slurred speech, dysphagia, muscle cramps, generalized muscle spasms and weakness. Central nervous system symptoms and signs include restlessness, anxiety, giddiness, vertigo, headache, confusion, stupor, coma and convulsions.

Neostigmine overdosage may produce practically all of these symptoms. However, when it occurs suddenly muscarinic symptoms and even fasciculations may be absent, and paralysis of muscles of deglutition and respiration may come on rapidly. This may resemble myasthenic crisis so closely that the desperate patient or physician may add even more neostigmine, with disastrous results. Cholinergic crisis is a greater threat to myasthenics who have especially weak pharyngeal and respiratory muscles, and to severe myasthenics with a fluctuating course who might fail to match a sudden remission with a rapid decrease in dosage.

In our series there are 186 severe or moderately severe myasthenics. This is the type of case in which crisis may be expected. Of these, from 1942 to December, 1954, twenty (10.8 per cent, thirteen females and seven males) have experienced crises, from which sixteen (8.6 per cent) failed to recover. Four have survived, three of them within the past two years. Duration of myasthenia before crisis ranged from six months to fifteen years, averaging 3.12 years. Onset of symptoms in practically all cases was rapid and severe, and in many the course of the disease was fulminating. All patients were receiving neostigmine at the time of crisis except one who was taking mestinon and the last three who were receiving WIN 8077.

Of the sixteen fatal cases, eight died suddenly. The remaining eight were treated in respirators from eighteen hours to twenty-one days. Two of these recovered from one crisis, only to die from a later one. Postmortem examinations were obtained in seven cases. In four, involuted thymuses were found. Two had small benign thymomas, one had an "involuted thymus with hyperplastic nodule." In only two were lymphorrhages found. In retrospect, it seems apparent that more than half of these fatal crises were of a cholinergic nature.

Of the four patients who recovered, three were treated in respirators; the fourth required only tracheotomy and suction. One of these patients was certainly in cholinergic crisis; in two this condition was probable. Myasthenic crisis developed in the fourth induced by 16 mg. of curare given at surgery to a previously undiagnosed myasthenic.

One of these patients, a twenty-four year old pregnant white woman, was hospitalized on December 8, 1952, for delivery. Her oral neostigmine dosage had risen to 215 tablets (3,225 mg.) daily. During an eight-hour labor she was given 4 mg. per hour parenterally. She was delivered of a vigorous 8 pound boy by mid-forceps. Thirty-six hours later he became obviously myasthenic. For ten days he required neostigmine but has not needed it since. Two days after delivery the mother suddenly stopped breathing. She became unconscious and deeply cyanotic but her pupils were miotic and her pulse was 80. Tracheal intubation was difficult as her jaws were powerfully clenched. She was placed in a respirator and given atropine, 1.2 mg. intravenously, and oxygen, with improvement. No neostigmine was given for twenty-four hours, during which time she continued to improve. Then she began to show increasing weakness which was relieved by small doses of neostigmine. She required the respirator intermittently for fifteen days. Since then her oral neostigmine dosage has varied between 50 and 100 tablets (750 to 1,500 mg.) daily. It would seem that this patient had a sudden partial remission after delivery but that her dosage was not reduced accordingly, thus precipitating a typical cholinergic crisis.

TREATMENT OF CRISIS

Whenever a myasthenic fails to clear secretions or to breathe adequately tracheotomy or tracheal intubation followed by tracheotomy should be performed. This insures an airway through which oxygen can be given and secretions can be aspirated. A respirator should be used without hesitation should voluntary breathing seem inadequate.

At this point an effort should be made to determine whether crisis is on a myasthenic or cholinergic basis. If excess secretions and/or miosis or relatively slow pulse are found, atropine 1.2 mg., should be given intravenously and repeated as long as these conditions are present. Although atropine will not relieve cholinergic weakness, it will alleviate most of the muscarinic symptoms. If no improvement occurs with atropine, tensilon® should be given according to the method of Osserman.[4] His most recent modification[5] consists of administration of 0.2 cc. (2 mg.) intravenously with the needle left in place. If improvement occurs, the crisis is probably myasthenic and the patient will undoubtedly respond to heavier dosage of cholinergic drugs. However, if muscle fasciculation appears, the crisis is probably cholinergic. Should no effect occur within thirty seconds, another 0.8 cc. is given. If muscle fasciculation and/or no improvement follow, cholinergic weakness should be suspected.

The course of one of our patients, a thirty-two year old Negro man who eventually died, illustrates the value of tensilon in differentiating myasthenic from cholinergic crisis. Two years previously he had survived a crisis. A year later another occurred, followed by frequent crises and intermittent psychotic episodes for seven months in which, without tensilon, it would probably have been impossible to keep him alive. During this time respirator care was repeatedly necessary. Because of his mental condition he was never able to differentiate underdosage from overdosage. We were able to do this with tensilon and his therapy was regulated accordingly, with reasonable accuracy.

Grob[2,3] has emphasized that in cholinergic crisis atropine tolerance and need is tremendous, and doses up to 2 mg. may and should be given intramuscularly at hourly intervals until signs of atropinization occur. He further advises,[8] and we agree, that cholinergic medication should be stopped until improvement occurs, followed by a return of weakness, at which time it should be cautiously resumed.

While the patient is in the respirator nothing should be given by mouth. Fluid and food requirements should be met by intravenous feedings, with a wary eye to fluid and electrolyte balance. However, as soon as the respirator is not constantly necessary, tube feeding may be used. Small amounts should be given frequently, and aspiration should be done before each feeding to guard against gastric retention. Antibiotic therapy should be started immediately and continued as long as a respirator is necessary, or as long as the patient coughs ineffectively. Should signs of atelectasis or any bronchial obstruction appear, bronchoscopy may prove to be life saving. Aranow[9] describes excellent results using mechanical exsufflation with negative pressure to prevent these conditions. He also warns against overventilation in the respirator and advises that CO_2 tension be checked frequently to avoid this hazard.

Cholinergic therapy should vary with the needs of the patient, and may be guided by

tensilon tests. Should any doubt exist as to adequacy of dosage, it is far better to underdose than to overdose. Mestinon® would seem to be the drug of choice for respirator patients because it produces less stimulation of flow of secretions and gastrointestinal activity. When cholinergic drug dosage requirements have stabilized, they may be given with intravenous fluids by continuous drip, as first recommended by Viets.[6] Atropine may be given as necessary to control excess secretions, but too much drying should be avoided. Morphine should not be given since cholinergic drugs potentiate its action.[3] Demerol® and barbiturates seem well tolerated, however, and are useful in allaying the extreme discomfort and anxiety present in crisis.

PREVENTION OF CRISES

In such a variable condition as myasthenia, rigid dosage schedules can never give adequate control. In no condition is it more important to teach each patient to vary dosage according to needs.

Patients should be taught to recognize cholinergic side effects and should be given atropine or belladonna to combat them should they occur. However, as emphasized by Schwab,[7] no severe myasthenic should ever take regular doses of atropine or belladonna to anticipate or prevent side effects without full knowledge of the danger of such a procedure. These drugs obscure or prevent such muscarinic symptoms as abdominal cramping, diarrhea, salivation and miosis. These are valuable signs of cholinergic overdosage; and, if present, warn the patient against taking more medication and thus precipitating cholinergic crisis. Unfortunately, muscarinic symptoms do not always appear before the onset of cholinergic crisis. However, patients who have experienced cholinergic weakness describe it as a peculiar "quivery" sensation, often accompanied by unusual dysphagia and dyspnea. Some patients also experience cramping or "tightness" of muscles.[8]

Because infection often precipitates crises, severe myasthenics should be supplied with an effective oral antibiotic for use at the first sign of infection.

In conclusion, it is this author's opinion that when drugs with more anticurare and less anticholinesterase activity become available, crises, both myasthenic and cholinergic, will become rare or non-existent in the treatment of myasthenia gravis.

SUMMARY

1. Crisis in myasthenia gravis is of two main types: True myasthenic crisis due to insufficient medication or drug resistance; and cholinergic crisis due to excess of cholinergic drugs.

2. Cholinergic crisis is a greater threat to myasthenics with especially weak pharyngeal and respiratory muscles, and to severe cases with a fluctuating course.

3. Differential diagnosis, treatment and prevention of crisis in myasthenia gravis are briefly presented.

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End of Symposium on Myasthenia Gravis

Clinical Studies

Acute Fatty Metamorphosis of the Liver Associated with Pregnancy*

A Distinctive Lesion

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ACUTE yellow atrophy of the liver is a literal translation of the German term, *akute gelbe Leberatrophie*, and it is construed as implying an illness of short duration and fatal outcome. The signs and symptoms of liver failure dominate the clinical picture, and jaundice is almost invariably present. The liver is usually found at autopsy to be smaller than average, soft, yellow, and to show microscopic evidence of acute lesions. Clinical entities such as subacute yellow atrophy and healed acute yellow atrophy have also been described; the implications of the terms in regard to the clinical features and the pathologic observations associated with them are obvious. Although one customarily associates acute yellow atrophy with fulminant epidemic viral hepatitis, this is not the only etiologic background for the development of the sequence of clinical events and anatomic findings. Similar phenomena have been noted in homologous serum jaundice (presumably caused by a virus somewhat different from that causing epidemic hepatitis), acute intoxications from arsenic, phosphorus, chloroform and carbon tetrachloride, Weil's disease (leptospiral hepatitis), and certain malnutritional conditions upon which some other additional (and unknown) factor is superimposed, such as kwashiorkor, etc. Certain experimentally induced dietary deficiencies produce a pathologic picture not dissimilar to that seen in kwashiorkor. Formerly, acute yellow atrophy of the liver used to be listed among the toxemias of pregnancy until it was recognized that most of such cases were merely fulminant epidemic hepatitis complicating pregnancy, and that the liver of pregnant women is damaged more readily and with greater severity by viral hepatitis than that of non-pregnant women. Frucht and Metcalfe[1] found that infectious hepatitis carried a higher mortality and a greater tendency to produce chronic liver damage when it occurred in pregnancy than when it occurred in non-pregnant women. Dill[2] reviewed the relevant literature and presented six fatal cases of acute yellow atrophy during pregnancy. He concluded that they were identical with severe infectious hepatitis. Implicit in his argument is the idea that acute yellow atrophy is an entity regularly equatable with severe forms of infectious hepatitis rather than a type of liver lesion which may develop following a variety of pathogenetic sequences. It is of some importance to note that it is generally possible to distinguish nosologically among these various etiologies of acute yellow atrophy by careful evaluation of the morbid anatomy of the liver. To this group of etiologic backgrounds may be added the "hepatorenal syndrome," an ill defined clinical entity in which death ensues after a short illness characterized by rapidly developing hepatic and renal decompensation. This syndrome embraces a wide variety of clinical phenomena and under its label is included a congeries of equivocal histopathologic changes.

In 1940 Sheehan[3] described a condition under the term "obstetric acute yellow atrophy" which he distinguished on histopathologic grounds from the more usual varieties of acute yellow atrophy. He was able to collect six cases of 400 autopsies on file at an obstetric hospital. Although he did not describe each case indi-

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vidually, his summation of the clinical and pathologic features is worth quoting in part. He states "Clinically the condition was very suggestive of true acute yellow atrophy. The usual course of the disease was as follows. At 36 to 40 weeks there was a sudden onset of severe vomiting and epigastric pain, followed in a few days by jaundice. These symptoms progressed very rapidly, the jaundice becoming very intense, the vomit was of coffee-grounds appearance, and headache was sometimes present. After 7–12 days the patient was delivered of a still-born foetus, became comatose, and died within three days after delivery. . . . At autopsy . . . the liver was yellow and rather small (1,040 to 1,300 grammes). Its lobular pattern was not lost. Microscopically all the cases showed an identical lesion: there was a gross fatty change affecting the entire lobule except a sharply defined rim of normal cells about the portal tracts. The affected cells were bloated by a fine foam of tiny fatty vacuoles throughout the cytoplasm, so that they resembled the cells of the suprarenal cortex. The nuclei were normal, and there was an entire absence of necrobiotic change . . . round cells throughout the fatty area. . . . The kidneys did not show any microscopic lesions, and in the two cases in which Sudan-stained sections were available, there was not any fatty change in the first convoluted segment."

Sheehan pointed out the paucity of reported cases in which this lesion was present prior to his publication. He cited the two cases described by Cullinan[4] in the appendix to his series of cases of "subacute necrosis of the liver" and the case reported in some detail by Stander and Cadden[5] as the only instances in which there was adequate documentation by pathologic examination of the presence of this distinctive lesion. Sheehan stressed the absence of necrosis as the most significant observation in his microscopic examination; whereas Stander and Cadden had indicated that they interpreted the fatty changes in the liver cells as a stage preceding necrosis, Sheehan pointed out that necrosis of parenchymatous organs which occurs as a result of slowly acting toxins is quite manifest histologically within two or three days, and that the patients with "obstetric acute yellow atrophy" survived for ten to fourteen days, exhibiting neither necrosis of liver cells nor any histologic indication that necrosis might have developed if they had lived longer. Sheehan also stated, "The pathological change in the livers of patients who die is of a reversible kind: if any of these patients had recovered, the liver cells could have returned to normal appearance within a few days. But, if a patient recovers, the diagnosis cannot be established with certainty, in view of the numerous other possible causes for jaundice."

Stander and Cadden present the details of the clinical and laboratory findings in a typical case of "obstetric acute yellow atrophy" stating that if, in the presence of persistent vomiting, dizziness and headache occurring in a pregnant woman, especially near term, icterus and drowsiness appear, it is almost certain that severe liver damage is present and that the prognosis is grave. The laboratory findings are those associated with severe, acute liver damage, irrespective of cause, and include increased serum bilirubin, decreased blood sugar, decreased blood urea nitrogen, increased blood uric acid, increased blood amino acids and decreased urea nitrogen in the urine. In regard to the pathologic observations upon the liver at autopsy, they point out that the liver cell nuclei are well preserved and that there is no frank necrosis to accompany the intracytoplasmic accumulation of fat within the liver cells.

Subsequent to Sheehan's report, Whitacre and Fang[6] presented a case of jaundice in pregnancy in which the microscopic appearance of the liver lesions as seen in two biopsies is identical with that described by Sheehan as well as by Cullinan and by Stander and Cadden. Their patient was a thirty year old Chinese, gravida II, para I, in whom weakness and anorexia developed followed by the onset of a respiratory infection, epigastric discomfort and vomiting of a "coffee-ground" material four weeks before term. She was icteric on admission, mentally dull, drowsy but conscious. She had moderate labor pains for four hours without progress, and her drowsiness increased to stupor. A cesarean section was performed and a live baby was delivered. The patient's liver was found to be of normal appearance and size, and a biopsy was performed. On the seventh postoperative day, a fit of coughing produced dehiscence of the abdominal incision with extrusion of loops of bowel. A secondary closure was performed and at this procedure the liver was seen to be smaller than before, distinctly yellow, and another biopsy was performed. The subsequent course was stormy, being complicated by another wound dehiscence, two profuse gastrointestinal hemorrhages, and shock. However, she responded to

treatment and recovered. Her jaundice diminished gradually and disappeared three weeks following hysterotomy. Tests of liver function showed impairment for five weeks after cesarean section. During the acute phase of her jaundice she had a low blood sugar and a low blood urea nitrogen, a transient increase in blood amino acids, a moderate elevation of blood uric acid and a low urinary nitrogen. These determinations confirm those described previously by Stander and Cadden.

Histopathologic interpretations in this case rest upon the two liver biopsies. The liver tissue removed at the time of cesarean section, when the hepatic damage was presumably maximal, showed well preserved lobular architecture. The liver cells in the central three-fourths or four-fifths of each lobule were swollen and clear, containing large amounts of fat and some clumped granular material near each nucleus. The cells at the periphery of the lobules were less clear, had less distinct cell borders, and were free of fat. A small amount of yellow pigment (presumably bile) was present in some of the central clear cells as well as in an occasional von Kupffer cell. A few lymphocytes and inconspicuous polymorphonuclear leukocytes were present in the portal spaces but the bile ducts were normal. There was no evidence of cell necrosis. The second biopsy, seven days later, showed substantially the same general features as the first except that the central clear zone of fat-containing cells was smaller, occupying only one-half of the diameter of the lobule, a slightly more conspicuous infiltration of polymorphonuclear leukocytes, lymphocytes and plasma cells, and the presence of mitotic figures in a zone lying between the central clear-cell area and the unaffected peripheral area. Whitacre and Fang were unwilling to accept this case as fitting into the group described by Sheehan, stating, "Sheehan reported 6 fatal cases very similar, if not identical, to this one as a separate entity apart from true acute yellow atrophy and suggested the name 'obstetric acute yellow atrophy.' We are not willing to do this on the basis of present knowledge, because the clinical course is identical, and the location and general type of lesion are the same. We may have to change our conception, but at this time it seems to be a matter of degree; that is, acute fatty degeneration of the liver is one stage in the process of necrosis. It seems reasonable to conclude that a dose of hormone, toxin, poison, infection, or combination of these factors could be insufficient to cause actual necrosis but still be sufficient to be usually fatal."

Of the six cases reported by Dill,[2] Case I (previously reported by Stander and Cadden) can be equated histologically with the cases described by Sheehan,[3] and Cases IV and VI present a comparable picture but cannot be assessed accurately because of the lack of a detailed report of microscopic observations.

It is of some value to note that LaDue, Schenken and Kuker[7] reported a case of phosphorus poisoning with recovery in which repeated aspiration liver biopsies were performed. The patient, a sixty-one year old white man, had swallowed an ounce of roach paste containing 2 per cent phosphorus and promptly developed signs and symptoms of acute intoxication with severe jaundice and other signs of severe liver damage. Aspiration biopsies were performed as late as the thirty-third, forty-fifth, eighty-sixth and 191st days after ingestion. Even as late as the thirty-third day areas of focal necrosis were present, and accumulation of fat in the form of both fine vacuoles and a clear type of cytoplasm were present in decreasing quantity as long as six months later. The earliest stages of healing by fibrosis were first seen in the biopsy at forty-five days after ingestion.

There is one other reported case which appears to conform in some respects to the group of cases isolated by Sheehan. Nixon, Egeli, Laqueur and Yahya[8] studied a group of twenty-one pregnant women, twelve with jaundice, in whom aspiration biopsies of the liver were performed. Their Case No. 20, a thirty-five year old primipara who developed pre-eclampsia at thirty-six weeks and gave birth twelve days later after medical treatment had rendered her normotensive and non-albuminuric, developed jaundice on the fourth postpartum day. A liver biopsy was performed at this time. Marked edema of the legs, hypertension and slight albuminuria developed the next day and she had three convulsions. On the sixth postpartum day she died with hyperpyrexia. At autopsy the liver weighed 860 gm., was of normal consistency and free from hemorrhage; the cut surface was tan and showed "small lighter stained areas evenly distributed." Microscopic examination of both the aspiration biopsy and the liver postmortem showed intact liver cell trabeculae and no necrosis. The liver lobules contained cells of two types: large polygonal cells with a clear

cytoplasm and regular, mainly vesicular nuclei, and large irregularly shaped cells with small nuclei and protoplasm very light and full of small vacuoles. Presumably, these cells contained fat and correspond to the cells described by Sheehan and the other authors cited. The presence of the antecedent unprovoked toxemia followed by apparent recovery, the recurrence of hypertension, albuminuria, edema and convulsions coincident with the onset of jaundice, make this case somewhat atypical in the small series of cases reviewed, hence difficult to evaluate. Nixon et al. considered the lesions as "change which could not be explained except by some kind of acute intoxication resulting in fatty degeneration. Such changes are seen in acute phosphorus poisoning. It is possible that this case was one of sudden functional breakdown of the liver owing to the strain of this particular pregnancy."

The three cases which serve as the basis for this article will illustrate certain of the clinical features of the course of this disease and are designed to serve as a point of departure for a discussion of certain of the pathologico-anatomic considerations which underlie an attempt to evaluate the disease process. Inevitably, certain questions will be asked which cannot be satisfactorily answered. It is hoped that such interrogations and speculations as are made will serve as a stimulus for further investigation and elucidation.

CASE REPORTS

CASE I. Mrs. E. B. (C.C.M.H. No. 34499), a twenty-four year old white, gravida II, para I, E.D.C. (expected date of confinement) about July 1, 1944, was admitted on May 14, 1944, because of nausea and vomiting of about two weeks' duration. These symptoms had been present over the entire period and had become more severe during the few days before admission. She noted that she had become jaundiced and estimated the onset of a yellowish tint to her skin as of about nine or ten days' duration. Jaundice had been slight at first but had become progressively more intense, and generalized pruritus had developed. These symptoms were accompanied by diffuse, poorly localized abdominal pain which was constant but not intense. The patient had noted that her stools had become extremely light, almost clay-colored, and that her urine had become very dark. Repeated questioning of the patient and her husband failed to elicit a history of poor diet, alcohol intake or exposure to possible liver toxins.

Physical examination revealed generalized icterus and dehydration. The abdomen was enlarged to a size consistent with a seven and one-half months' pregnancy, with the fetus in L.O.A. position. Soft distention and diffuse tenderness, more marked in the right upper abdominal quadrant, were observed. The liver edge and spleen were not palpable. There were no ecchymoses or purpuric manifestations. There was no suprapubic pain or tenderness.

On admission her temperature was 98°F., pulse 96, respirations 20, blood pressure 118/74. The admission urine specimen had a specific gravity of 1.017, was dark amber, acid, free from sugar or acetone, contained bile and a trace of albumin. The hemoglobin was 88 per cent, red blood count 4.73 million, white blood count 19,500 with 87 per cent polymorphonuclear leukocytes, 12 per cent lymphocytes and 1 per cent monocytes. A rare normoblast was found. The icterus index was 100. Examination of her vomitus was positive for occult blood and bile. The patient was treated with intravenous fluids, vitamin B complex, vitamin K, and given a high carbohydrate, high protein diet.

The day following admission she went into spontaneous labor and was delivered rapidly, almost precipitately of a 5 pound, 15 ounce girl. The membranes ruptured during the second stage of labor. No lacerations were incurred at delivery. A moderate amount of postpartum bleeding occurred, which ceased after injection of 3 ampuls of ergotrate.®

The day following delivery the hemoglobin was 62 per cent, red blood count 3.13 million, white blood count 32,000 with 77 per cent polymorphonuclear leukocytes, 5 per cent lymphocytes and 2 per cent monocytes, and a few nucleated red cells were found. Again the vomitus was positive for occult blood and bile. The same day the urine was still dark amber and acid, had a specific gravity of 1.020 was free from sugar or acetone, but was positive for bile, and had a trace of albumin. Following delivery her condition became worse. Vomiting was more severe and jaundice increased. She was treated with transfusions of compatible blood, Wangensteen suction, intravenous glucose solutions, as well as supplementary vitamin B complex and vitamin K. She became stuporous and irrational. Her temperature rose to 105.4°F., pulse 150, respira-

tions 40 and shallow, and she died on the fourth postpartum day.

At birth, the infant appeared to be in good condition but respiratory difficulties soon supervened necessitating vigorous treatment for what was diagnosed as "atelectasis." Although the infant recovered from this episode and is now living (age ten), she exhibits signs of mental retardation and emotional disturbance.

Gross observations (B.L-i.H. No. A-44-23): The postmortem examination was limited to the contents of the abdomen and pelvis. The most significant findings were in the liver and kidneys. The uterus was compatible in size and configuration with a four days' postpartum appearance, and there was no failure of the placental site to heal. Between 3,000 and 4,000 cc. of bile-stained fluid were present in the peritoneal cavity. There was moderate to marked edema of the duodenum and jejunum. The retroperitoneal tissues were also edematous and the lymph nodes in this area were enlarged, soft and juicy. The gallbladder was not thickened and contained no calculi; bile was easily expressed into the duodenum along the biliary tree, and dissection of the biliary tree revealed no obstruction. The ampulla of Vater was located in the third part of the duodenum rather than the second. The liver was not remarkable in shape but no weight is recorded. However, the liver edge was rounded. The capsule was thin, translucent and tense. On external inspection the liver markings were moderately distinct and the color varied from reddish gray to putty-colored. The cut surface showed increased prominence of the liver markings, and was pale greenish brown with a yellowish cast. No gross evidence of intrahepatic biliary obstruction was found. The bile in the smaller biliary ducts was dark, concentrated and mucoid.

The kidneys weighed approximately 110 gm. apiece. The capsule was somewhat thicker than usual but stripped with ease revealing a peculiar, congested, somewhat icteric capsular surface. Sagittal sections showed the usual gross renal architecture. The cortex was strikingly pallid and measured 7 mm. The medullary markings and striations were not remarkable. The mucosa of the pelvis was congested and the pelvis contained a small amount of turbid, icteric urine.

Microscopic observations: Sections of the liver stained with hematoxylin and eosin showed that the lobular architecture was well preserved insofar as the spatial relationship of central veins to portal areas was concerned but the individual lobules were swollen, compressing the interlobular boundaries so that the zone between lobules was indistinct. With the exception of a discontinuous thin rim of apparently normal liver cells at the periphery of the lobule, the entire lobule was composed of swollen vacuolated liver cells. (Fig. 1A.) The parenchymal cells were arranged in the usual trabecular pattern, and the relationship of liver cords to sinusoids was preserved. The conspicuous change was in the cytoplasm of these cells; almost every cell contained a myriad of fine vacuoles which were separated from each other by delicate eosinophilic cytoplasmic strands. (Fig. 1B.) Coalescence of vacuoles was not a prominent feature, and large single vacuoles pushing the nucleus to one side, producing a signet ring appearance, were not seen. The nucleus was almost invariably in the center of the cell. The nuclei appeared slightly smaller than average but this may have been more apparent than real because of the increased cytoplasmic area. The nuclei were well preserved, with a distinct chromatin pattern, were not pyknotic or fragmented, and appeared viable in every respect. Binucleated liver cells were found in quantity. The von Kupffer cells were not increased in number; a few of them exhibited a yellowish tint to their cytoplasm, presumably due to accumulation of intracytoplasmic bile pigments. Here and there a few small sprinklings of infiltrating inflammatory cells were present. For the most part these were lymphocytes but an occasional plasma cell was present among them, and a rare polymorphonuclear leukocyte could also be seen. This minimal infiltrate was not localized to any portion of the lobule; it was no more prominent in the portal spaces than in its irregular distribution in the sinusoidal spaces. Conspicuously absent was any degree whatsoever of necrosis of liver cells. Reticulum stains showed an intact fibrillar architecture to the lobules, confirming the impression gained from study of hematoxylin and eosin sections. A Hotchkiss-McManus stain failed to demonstrate mucopolysaccharides and glycoproteins within the cytoplasm of the altered liver cells. It is inferred from the size, shape and transparency of the vacuoles that they represent intracytoplasmic lipid within the cells.

The general architecture of the kidney was well preserved. The glomeruli were somewhat more plump than usual, and there was some prominence of the capillary loops as well as

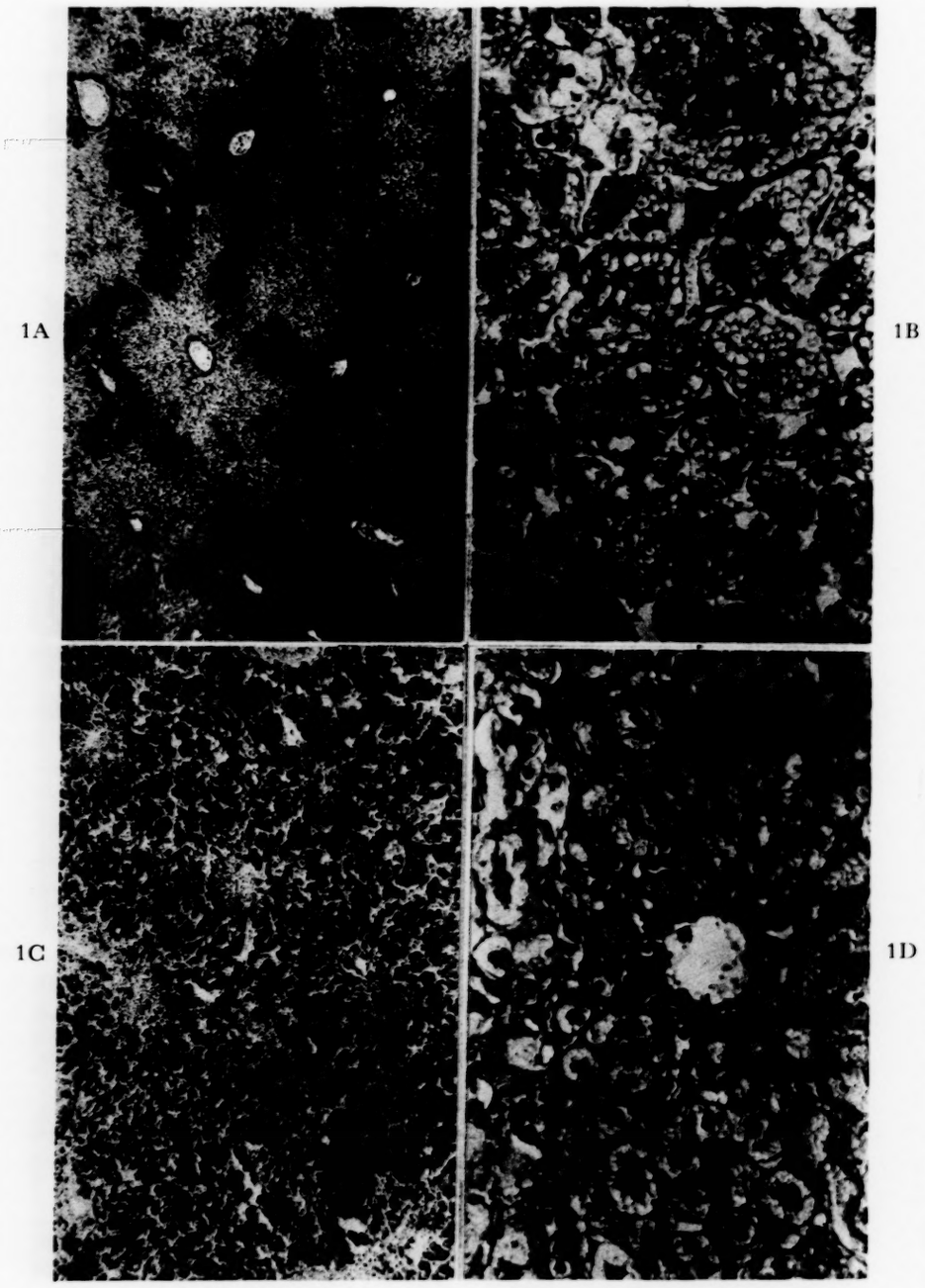

FIG. 1. A, Case I. Low power view showing pale areas of fatty metamorphosis in the central two-thirds of the lobule surrounded by a darker rim of spared liver cells; hematoxylin and eosin stain, liver. B, Case I. Detail of liver cells with fatty metamorphosis showing multiple fine vacuoles distending the cytoplasm of the cells. A binucleate cell is shown; hematoxylin and eosin stain, liver. C, Case II. Low power view showing distribution of fat in the liver lobules; oil red O stain, liver. D, Case II. Detail showing central vein with slight paracentral necrosis and a surrounding zone of fat-filled cells with multiple tiny vacuoles honeycombing the cytoplasm; hematoxylin and eosin stain, liver.

equivocal increase in the amount of the glomerular interstitial substance. However, the most striking changes were in those tubules which lay within the medullary rays of Ferrein. The epithelium lining these tubules was pallid, and the cytoplasm of the epithelial cells finely foamy or vacuolated. (Figs. 2B, C.) The change was sharply localized to this area for it was not present in the upper portion of the proximal or in the distal convoluted segments which lay elsewhere in the cortex. The nuclei of cells so affected were indistinct and their chromatin stained poorly; whether or not this is true necrobiosis is difficult to state with certainty. No lesions were seen in the renal vasculature and the medulla was unremarkable. Although formalin-fixed tissue was not available for Sudan stain, the alteration was interpreted as compatible with fatty accumulation within the cytoplasm.

The pancreas was histologically normal, apart from slight interstitial edema. No necrosis was noted.

The final anatomic diagnoses were fatty metamorphosis, liver, acute, massive; fatty metamorphosis, kidneys, medullary rays of Ferrein; ascites; lymphadenitis, retroperitoneal nodes; icterus, generalized: status postpartum, uterus.

Case II. Mrs. T. C. (M. H. No. B3243),* a twenty-six year old primipara due to deliver on October 20, 1952, was admitted to the Milton Hospital on October 8th. She had had a completely uneventful prenatal course with no sign of elevated blood pressure or other difficulty. Previous health had been excellent. No history of dietary deficiency or of exposure to liver poisons was elicited at this time or on subsequent questioning of her husband. She was group O, Rh positive. On October 6th she complained of severe headache, with no other signs. Her blood pressure was then 120/80. The urine was negative. She was given codeine and empirin without relief. On October 7th the headache was still present and she vomited. She was then sent to the hospital where her temperature was found to be 100.6°F., pulse, 100, respirations 20, blood pressure 128/80. She was given 300,000 units of penicillin. The fetal heart rate was found to be 136. The urine was reported negative. On October 9th the temperature was normal but the white blood count was 17,000 with three nucleated red cells. The mother's heart rate was now 120. The fetal heart was not heard, possibly due to the loud action of the mother's heart. A lumbar puncture was done which yielded clear fluid with no cells. On October 10th the patient was semi-comatose but was rational when roused. The blood pressure was 120/80. The pulse was 100. The urine now showed a 1+ albumin and fairly numerous hyaline and granular casts, as well as ten to twelve white blood cells per high power field. The leukocyte count in the blood was 20,400. The uric acid was 5.6 mg. per cent. The serum NPN was 74 mg. per cent. On the following day she was found to be definitely jaundiced. She was in active labor in the morning and was semi-comatose, with blood pressure of 120/80. She was delivered by low forceps of a dead female fetus which was not macerated but showed a soft skull. After delivery there was persistent oozing and it was found necessary to pack the uterus. Coma deepened and several hours later the patient died. The bilirubin was found to be 6.2 mg. per cent total, with 3.8 mg. per cent direct. The serum NPN on that day was 72 mg. per cent. The chlorides were 95 mEq./L. The thymol turbidity was four Maclagan units. Prothrombin concentration by Quick's method was 11 per cent.

Gross observations (F. H. No. U-52-32): Postmortem examination was limited to the thorax and abdomen. The body was well developed and well nourished. A thin, brown fluid trickled from the corners of the mouth. Each pleural cavity contained 2 or 3 cc. of clear fluid. The heart weighed 300 gm. and was not unusual in any way except for a small subendocardial hemorrhage in the left ventricle. The lungs weighed 340 and 310 gm., respectively, and showed a few small scattered elevated red-brown spots evidently due to terminal aspiration of brown "coffee-ground" fluid, which was observed in the mouth, stomach and bronchi. The peritoneal cavity contained about 15 cc. of bright red bloody fluid which had apparently oozed through from the parametrium. The gastrointestinal tract was remarkable only for the presence of pale, almost clay-colored feces in the colon; no ulcers were found. The only other significant findings in the abdomen involved the uterus, liver and kidneys. The uterus showed the expected postpartum appearance, with the addition of rather extensive hemorrhage into the parametrium on both sides. The liver was decidedly shrunken, weighing only 810 gm. The capsule was thin and

* The authors are grateful to Dr. Augustine Rogers for permission to present the clinical history of this case.

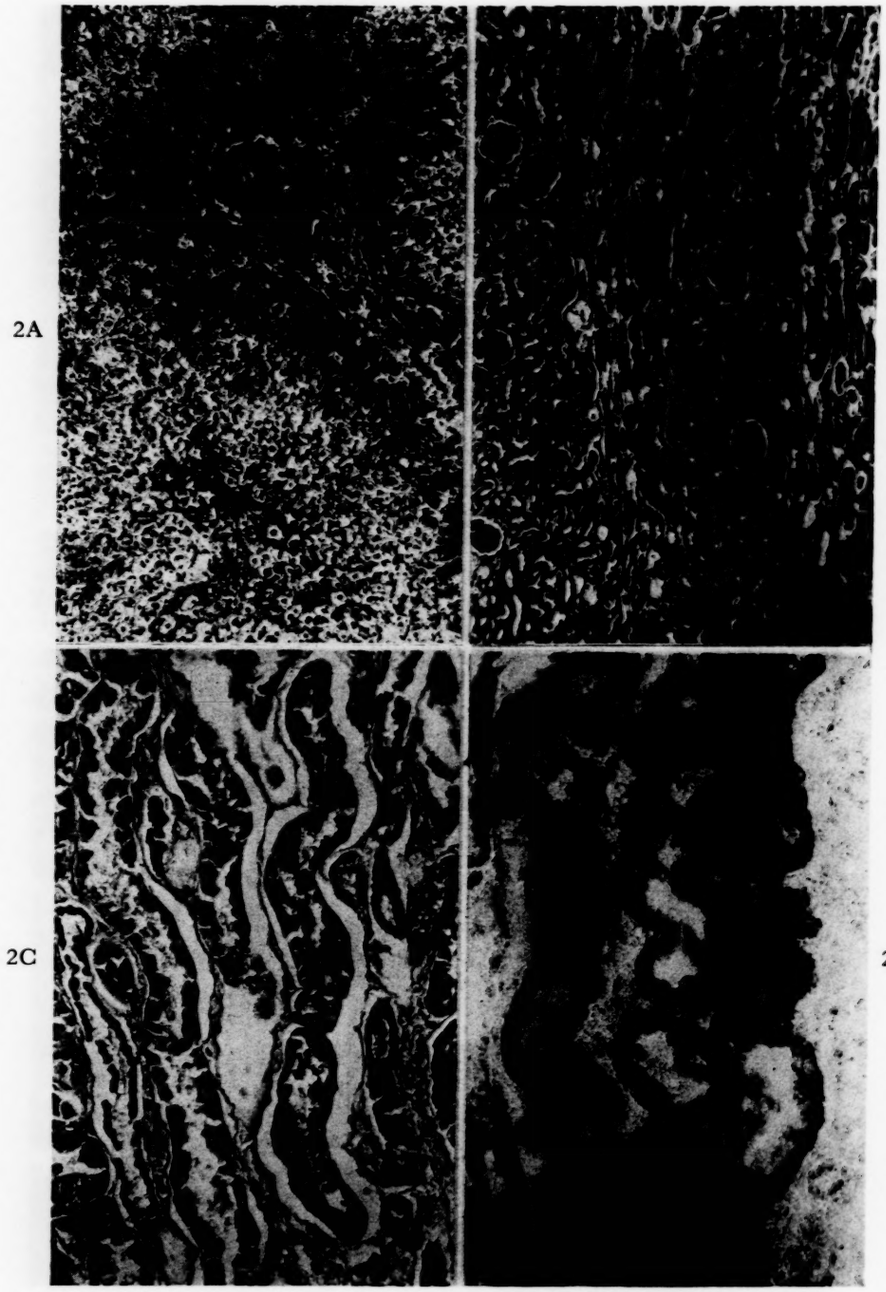

FIG. 2. A, Case II. Detail showing the peripheral spared area near the portal space in contrast with the central fat-filled area of the lobule; Gomori trichrome stain, liver. B, Case I. Low power view showing pale staining of the columns of Ferrein contrasting with more intense staining of the convoluted segments; hematoxylin and eosin stain, kidney. C, Case I. Detail of tubules in the column of Ferrein showing pale staining interpreted as fine fatty droplets; hematoxylin and eosin stain, kidney. D, Case II. Detail of proximal convoluted tubules showing fine fatty droplets within the cytoplasm of epithelial cells; oil red O stain, kidney.

smooth but had a tendency to wrinkle. Externally the liver was flabby and had a dull yellow-pink color. On section the tissue seemed more friable than usual, and the color was a dull pale yellow with numerous scattered minute red spots corresponding to the lobular markings. There was no difference between right and left lobes, and there was no obstruction of the intra- or extrahepatic vessels or bile ducts. The gallbladder was small, containing 3 or 4 cc. of viscid green bile with no calculi. The common bile duct was not dilated. The kidneys weighed 130 and 160 gm., respectively. They were unusually pale, probably because of blood loss. The capsules stripped easily from each other, revealing a pale yellow-pink surface which was smooth except for a few scattered shallow depressions measuring from 0.2 to 0.8 cm. in diameter. On section the cut surface showed a slightly swollen appearance with a yellowish cast on a pinkish tan background, but cortex and medulla were well defined, and some of the cortical striations could be seen. The pelves, calyces and ureters were not dilated and were lined by an intact pale mucosa except for a few petechial hemorrhages in one renal pelvis. The placenta weighed 450 gm. and was not remarkable. Permission for fetal autopsy was not granted.

Microscopic observations: Aside from a moderate chronic gastritis, significant findings were confined to the liver and kidneys.

The liver closely resembled that of Case I in that all the hepatic cells, apart from a narrow well preserved zone around the portal areas, were filled with cytoplasmic vacuoles, several to each cell. (Fig. 2A.) Fat stains confirmed the lipid nature of these vacuoles and that several were present in each cell, in contrast to the single, signet ring appearance often seen in fatty livers. (Fig. 1C.) Although the affected cells were clearly swollen and larger than usual, and no frank necrosis was seen, it was obvious that the portal zones and central veins were closer to one another than they are in the normal liver. This implies a decrease in site of the lobules due to loss of liver cells, as indeed must have been the case in view of the small size of the liver. Perhaps to be correlated with this fact was the observation of groups of cells containing fine yellow pigment granules, situated near the central veins, usually in the company of a few lymphocytes and occasional polymorphonuclear leukocytes. (Fig. 1D.) These groups of cells appeared to lie within spaces which at first sight appeared to be the usual blood sinusoids. However, erythrocytes were usually not present and an endothelial lining was not often demonstrable. While many of the pigmented cells had reniform nuclei, others had round or oval nuclei, and it is conceivable that some of them might be bile-stained dying liver cells, although certainly many are phagocytes. Another finding, not prominent in Case I, was an abundance of bile "plugs" in the canaliculi of the central and mid-portions of the lobules. Thus an obstructive element is added in this instance.

The kidney showed a few small subcortical scars, associated with rather heavy infiltration of lymphocytes and apparently representing a healed focal pyelonephritis. Otherwise the renal parenchyma, in the hematoxylin-eosin stain, was not remarkable except for a few vaguely defined vacuoles in the proximal convoluted tubules and a little granular precipitate in the lumina of these tubules. The fat stain, however, showed an abundance of fat droplets in the proximal convoluted tubules and in the collecting tubules of the medulla, but not in the distal convoluted tubules. The fat in the proximal tubules was arranged in relatively small but numerous globules in the basal parts of the cells. (Fig. 2D.)

The placenta was not remarkable. It showed fairly numerous foci of calcification in the intervillous fibrin deposits but no necrosis or vascular lesions.

The final anatomic diagnoses were fatty metamorphosis, liver, massive, acute; fatty metamorphosis, renal tubular epithelium; aspiration of gastric contents, agonal; status postpartum, uterus.

CASE III. Miss G. B. (F. C. H. No. 1443),* a sixteen year old primigravida, was admitted to the Florence Crittenton Hospital on August 8, 1928, at seven and one-half months' gestation. Her last menstrual period had been on December 20, 1927, and her E.D.C. was September 27, 1928. She was in good health when admitted and remained so until September 8th when she began to vomit. She was sent to bed and was given an enema on the following day. Her vomiting ceased but she spent both September 9th and 10th in bed, acted queerly, talked

* This case is reported through the courtesy of Dr. G. Kenneth Mallory, who recalled having performed the autopsy when the initial manuscript was given him. Dr. Mallory graciously placed this case at the authors' disposal and prepared additional sections for study.

irrationally and became somewhat unresponsive. At 4:00 A.M. on September 11th she was found sitting on the floor counting aloud. She was put back to bed, making no resistance and went to sleep. At daylight she was noted to be jaundiced and quite irrational. At 8:20 A.M. her membranes ruptured spontaneously and she went into good labor promptly. A slightly macerated stillborn male infant weighing 6 pounds was delivered at 10:50 A.M. and the placenta at 11:45 A.M. The placenta was small, yellowish and hard. While repairing a small perineal tear, it was noted that the patient was in poor condition with a pulse of 120. She was given a subcutaneous saline infusion, stimulants, etc., but failed rapidly and died at 12:05 P.M. No urine had been obtained by catheter during delivery.

Gross observations (Mallory Institute of Pathology No. U-28-36): The body was that of a well developed and well nourished girl, 157 cm. long. The skin and scleras were icteric. Slight rigor mortis but no lividity or edema was present. The liver was smaller than average (not weighed), and extended 5 cm. below the xiphoid and 2 cm. below the costal margin in the midclavicular line. The external surface was smooth and pale yellow with superficial capillary injection. The substance of the liver was firm and the cut surface was homogeneous and yellow. There was no evidence of obstruction in the gallbladder or bile ducts. The kidneys were normal in size and pale yellow brown. The capsule peeled easily revealing a smooth surface. The cut surface showed an even cortex 0.5 cm. thick. The renal pelves showed no lesions. The uterus was compatible in appearance with a recent postpartum state. The other viscera were grossly unremarkable.

Microscopic observations: As in the two previously cited cases, the pathologic changes were confined to the liver and kidneys. Sections stained with phloxine-methylene blue showed that the lobular architecture of the liver was well preserved and that no necrosis was present. The liver trabeculae were composed of well preserved cells with extensive vacuolation of their cytoplasm, the vacuoles being rather small, many being present in each cell, producing a coarsely foamy appearance. The nuclei of these cells were centrally located and their chromatin was distinct and well preserved. Occasional binucleate cells were seen. This vacuolation occupied almost the entire thickness of each lobule, there being only a thin rim of spared liver cells adjacent to each portal space. The vacuolated cells were ballooned and the sinusoids compressed. There was no evidence of bile stasis but occasionally bile pigment was seen in von Kupffer cells. A scattered and inconspicuous infiltration of lymphocytes and an infrequent plasma cell were found sporadically in portal spaces and occasionally in lobules. Formalin-fixed tissue was not available for fat stains.

The kidneys showed the usual microscopic architecture. The glomeruli were well preserved and free from infiltration or scarring. Examination of the epithelium of the proximal convoluted segment revealed the cytoplasm of many cells to contain small vacuoles. The nuclei of these cells were well preserved and stained sharply. In the medullary rays of Ferrein the tubular epithelium became pale and eosinophilic, and nuclear staining was indistinct. However, in these cells also there was fine vacuolation of the cytoplasm. Although tissue was not available for fat stain, this was interpreted (as in Case 1) as a combination of fatty metamorphosis and postmortem autolysis. No changes were seen in the blood vessels or interstitial tissue of the kidneys.

The final anatomic diagnoses were fatty metamorphosis, liver, acute, massive; fatty metamorphosis, renal tubular epithelium; status postpartum, uterus.

COMMENTS

The clinical aspects of these cases seem to be reasonably well defined. The clinical course is characterized by the onset of jaundice late in the third trimester of pregnancy. Hemolytic and obstructive jaundice can be ruled out. The disease is clearly one of the varieties of parenchymatous (hepatocellular) jaundice. The signs, symptoms and laboratory in disations of acute liver failure develop. The patients become progressively worse even after delivery and die in hepatic failure. On purely clinical grounds the disease is not distinguishable from fulminant epidemic hepatitis. Of the fourteen cases reported (including the present three), thirteen cases have been fatal, a mortality rate of 92.9 per cent. Only the case reported by Whitacre and Fang recovered. Whether this is an isolated instance or whether a number of the cases diagnosed clinically as "acute yellow atrophy" but not confirmed by autopsy were in reality this type of lesion, or whether the disease does occur in a less fulminant form in which there is

recovery (with or without residual fibrosis) are matters for speculation. It is doubtful whether the diagnosis can be made antemortem without morphologic confirmation from a liver biopsy.

Likewise, the pathologic findings in these cases are reasonably consistent. There is diffuse fatty metamorphosis of the liver with preservation of lobular architecture. The peripheral portion of the lobule is spared. There is either no evident necrosis or merely minimal paracentral necrosis, albeit there is evidence of loss of cells in Case II. There is either no infiltration by inflammatory cells or else a sparse, randomly distributed infiltration of a few lymphocytes, an occasional polymorphonuclear leukocyte and a rare eosinophil. There may or may not be fatty metamorphosis of the renal tubular epithelium. On purely histopathologic criteria, the lesion in these cases cannot be classed as infectious hepatitis. It resembles more closely the effects of an exotoxin or an endotoxin upon the liver.

While this manuscript was being prepared for publication, a fourth case of this clinical syndrome associated with this distinctive liver lesion was brought to our attention.* The patient, a twenty-nine year old woman, died forty-three hours after delivery of a stillborn infant, having been ill for one week prior to the onset of labor. Postmortem examination revealed an acute hemorrhagic pancreatitis in addition to the central multilocular fatty metamorphosis of the liver without necrosis. The combination of these two lesions was reminiscent of those seen in experimental ethionine intoxication.

A body of experimental evidence has recently been accumulated that ethionine, a chemical analogue of methionine which enters into metabolic competition with it, can produce necrotizing pancreatitis and fatty metamorphosis of the liver cells. Goldberg, Chaikoff and Dodge[9,10] noted that the pancreatic lesion was restricted to the acinar tissue and that the islets of Langerhans were spared. Farber and Popper[11] were able to prevent the appearance of the liver and pancreatic lesions by simultaneous administration of methionine, but not by cysteine or glucose (which latter does prevent the liver lesion). Stekol and Weiss[12] observed that smaller doses of ethionine inhibited the growth of mice but that this inhibition wss reversed by methionine or choline, but not by cysteine or homocysteine. Wachstein and Meisel[13] found that the effects of ethionine were enhanced by preliminary dietary protein depletion. Simpson, Farber and Tarver[14] demonstrated that ethionine affects protein synthesis by inhibiting the incorporation of methionine and glycine into protein in the liver. Bollag and Gallico[15] showed alterations in enzyme activity even before histologic changes appeared; pancreatic diastase was reduced and then abolished; pancreatic catalase was somewhat increased; liver catalase activity was conspicuously depressed. The syndrome of ethionine intoxication resembles closely kwashiorkor; Brock and Autret[16] have suggested methionine deficiency as one of the possible primary etiologic factors in the development of kwashiorkor. Jensen, Chaikoff and Tarver[17] found an increased total lipid content in the livers of fasted female rats to whom ethionine had been administered. This increase was in the fatty acid moiety, there being no increase in the cholesterol or phospholipid fractions. A control series of fasted male rats also given ethionine failed to demonstrate increased lipids.

Following the procedures of Jensen and his colleagues, chemical determinations of the lipid contents were done on formalin-fixed material from Case II. It must be noted that Jensen et al. used fresh liver, but this was not available to us, and may limit the accuracy of the quantified data. Using 5.49 gm. of formalin-fixed liver, it was determined that total lipids were 13.04 per cent, total fatty acids 10.8 per cent, phospholipids 0.23 per cent, total cholesterol 0.19 per cent, free cholesterol 0.14 per cent and cholesterol esters 0.05 per cent. This corresponds closely with the values given for hepatic lipids in ethionine-treated fasted female rats. It is clear that in both instances there is an increase in liver fat due to an increase in fatty acids.

It is of some interest that in these cases the intracellular lipid appears quite regularly as fine droplets which give a honeycombed appearance to the cytoplasm. The more usually found large, single, intracytoplasmic vacuole which pushes the nucleus off to one side is conspicuously absent in these cases. Whether this is an artefact of the rate of intracellular lipid accumulation or whether it is a specific property of fatty acids in contrast to neutral fats seems to be a matter regarding which one can merely speculate.

Another feature of this type of lesion which must perforce lead to speculation is the problem of the liver weight. Apparently, there is con-

* This case will be reported in detail by Dr. Aaron W. Newton and Dr. Harwood Cummings.

siderable range in the weight of the liver at autopsy in these cases—from 810 to 1,300 gm. In view of the fairly obvious observation that the liver cells are individually increased in size, and that there is no necrosis of liver cells, does this imply a disappearance of liver cells without trace? In fact, are the liver cells numerically reduced, that is, is this a true atrophy? Measurement of the radii of lobules and of the distances from one portal space to another fail to show decrease in the size of lobules. By inference, the fact that individual liver cells are enlarged indicates that there are fewer cells per lobule. The specific gravity of the fat-laden cells is lower than that of normal liver cells. Presumably, the decrease in liver weight can be ascribed largely to the accumulated lipid.

Parenthetically, the occasional observation of paracentral necrosis near an occasional central vein need not be construed to indicate that a necrotizing agent (viral, bacterial or chemical) is etiologically implicated. Glynn and Himsworth[18] have demonstrated that the intralobular circulation is greatly restricted during the phase of acute fatty metamorphosis because the swollen liver cells narrow the sinusoids, thereby favoring ischemic necrosis in and about the centrilobular zone.

A question that will naturally be raised is whether one of the halogenated hydrocarbons (e.g. carbon tetrachloride) might have been involved in either or both of the cases, since these agents are known to produce fatty livers and renal damage. Aside from the fact that persistent questioning of relatives revealed no exposure in either patient, it may be suggested that the lesions in both liver and kidney would be expected to be much more severe if these drugs played a role. Williams[19] points out that in moderately advanced carbon tetrachloride intoxication following the administration of a single dose to mice there is extensive central necrosis, but that in early carbon tetrachloride intoxication the changes resemble those seen in starved mice in which there is no necrosis. His detailed analysis of the cytoplasm of affected cells showed increased lipid, decreased protein, decreased glycogen, as well as increased alkaline phosphatase, decreased ribonucleic acid, arginine, tyrosine or histidine. He points out that these intracellular changes are reversible four to six days after the single injection of the exotoxin. Williams also noted that in irreversibly damaged (i.e. necrotic) liver cells a PAS-positive polysaccharide-protein complex was present. This was not found in any of the three cases herein reported and further confirms statements regarding the absence of necrosis and the reversibility of the lesion.

The renal lesions in these three cases furnish evidence that the disease process is not confined to one organ. In Case I, degenerative changes were noted to be sharply limited to the columns of Ferrein, presumably involving only the terminal portion of the proximal convoluted segment. The lesion consisted of alteration of the tubular epithelium; the cytoplasm of the cells was finely vacuolated and somewhat more eosinophilic than elsewhere in the tubular epithelium. Also, the nuclear outline was indistinct and nuclear chromatin did not stain as sharply as elsewhere. Whether these observations add up to fatty degeneration without true necrosis or whether true necrosis of the epithelial cells in this segment of the tubule was present is difficult to assess. In Case II the lesion in the tubular epithelium was less sharply localized; there was fine fatty vacuolation of many parts of the proximal convoluted segment; no necrosis was evident. There is some evidence from experimental sources that the concatenation of the liver lesions seen in these cases with changes in the renal tubules is not entirely fortuitous. Farber, Simpson and Tarver[20] noted fat in the epithelium of the convoluted tubules in fasted female rats fed ethionine. Wachstein and Meisel[21] observed that normal rats when fed ethionine would develop necrosis of tubular epithelium limited to the terminal portion of the convoluted segment; they also observed that, like the liver lesions, the renal lesions were increased in severity if the animals were placed upon a protein-depleted diet and that the lesions were prevented by administration of methionine. Wachstein and Meisel pointed out that the renal lesion was similar to that produced by administration of *d,l*-serine. This type of experimental evidence implies that in the two clinical cases described there may be some comparable process of biologic antagonism or competitive metabolism which underlies the development of the lesions. Parenthetically, both Wachstein and Meisel as well as Alvizouri and Warren,[22] who confirmed their observations, indicate that the lesion of the renal tubular epithelium is reversible in experimental animals.

The occurrence of these cases exclusively in the last trimester of pregnancy raises the inevitable

question: In what way is the development and nature of the lesion related to the pregnant state? Is there anything specific about the pregnant state which would favor the development of such a lesion? A certain amount of indirect evidence may be adduced regarding this matter. In general, the normal liver is able to function throughout pregnancy without overt signs of functional or morphologic failure. Ingerslev and Teilum[23] performed needle biopsies of the liver in healthy pregnant women and found no anatomic evidence to support the concept of "Schwangerschaftleber"; because of this observation as well as innumerable reports of normal liver function tests during pregnancy, the concept has generally been discarded. On the other hand, there are certain clinical observations which indicate a "lowered hepatic reserve" during pregnancy. Just what this "reserve" is seems difficult to define, for the essence of liver function is that there is one type of cell which apparently performs all the innumerable metabolic functions of the liver. There is, however, a quantitative limit to the ability of the liver cells to function, either as a unit or in aggregate. For example, the spider angiomas and liver palms which are frequently found in patients who suffer from liver disease are frequently seen in healthy pregnant women. The appearance of these peripheral phenomena is ascribed to the inability of the liver to metabolize estrogens at the rate at which they are being produced. The comparison of estrogen inactivation by the diseased liver in the presence of normal estrogen production or the hyperestrogenism of pregnancy leads one to the generalization that a given unit weight of liver tissue (or a given number of liver cells) can inactivate a given quantity of estrogen in a given period of time. This principle can be applied to any of the other functions of the liver cell.

Biskind[24] has summarized the relation of nutritional deficiency to inactivation of estrogen in the liver, and has shown that the ability of the liver cell to perform this one function depends upon the maintenance of adequate levels of certain vitamins. He points out that "impairment of the estrogen-inactivating mechanism of the liver occurred in the absence of detectable morphologic change in this organ; conversely, inactivation of estrogen can occur in livers which are the site of severe necrosis and fat infiltration. . . . The functional and morphologic changes in the liver thus bear no necessary relation to each other." This observation is applicable in greater or lesser degree to any individual function of the liver cell one may care to investigate. However, Himsworth and Glynn[25,26] were able to produce two separate and distinct types of liver damage in rats. By utilizing a diet low in proteins they were able to produce massive hepatic necrosis which, although often fatal to their experimental animals, did on occasion heal with the production of "postnecrotic scarring." Utilizing a diet which contained either an excess of fat or a deficiency in lipotropic factors, such as choline, they were able to produce extensive fatty infiltration of the liver as well as diffuse hepatic fibrosis. Subsequently, they demonstrated that a diet deficient in protein but containing adequate amounts of methionine would prevent the development of massive hepatic necrosis. Handler and Dubin[27] confirmed the observations that a choline deficient diet would produce fatty infiltration and diffuse fibrosis of the liver, and demonstrated that either thiamine deficiency or restricted food consumption would prevent the accumulation of excessive liver fat. Subsequently, Glynn[28] observed that "considering the nutritional priority that nature grants to the developing fetus, it is not surprising that pregnancy tends to reveal states of latent malnutrition. A dietary level of thio-amino acids barely sufficient for a nonpregnant woman would become grossly deficient in the presence of pregnancy. . . . Development of a dietetically induced yellow atrophy in pregnancy necessitates a nice balance between the amount of vitamin E necessary to maintain pregnancy and the amount required to maintain the integrity of the liver, too much preventing necrosis and too little preventing pregnancy." Presumably this applies only to the acute liver necrosis in rats which can be induced by thio-amino acid deficiency and prevented by adequate vitamin E intake. However, there are probably additional variables in the diet of pregnant women which, acting either singly or in concert, are in nice equilibrium between liver necrosis and pregnancy failure. It is not unreasonable to infer that as pregnancy progresses, as the fetus develops, and as its metabolic and nutritional needs increase, the margin between the requirements of a given nutrient and an insufficiency of that nutrient becomes progressively narrowed. As the threshold of insufficiency is approached the homeostatic mechanisms

which control the sum total of liver functions are maintained at progressively changing quantitative equilibria; when equilibrium cannot be maintained either because of decreased intake, excessive demand, or both, functional failure of the liver may ensue. This may or may not be accompanied by anatomic changes in the liver cell. Presumably, if functional failure is present to any significant degree, especially in regard to certain of the more important functions, and for any significant time, there will be some morphologic evidence that such has been the case. Put more simply, the pathologic changes may occur a little later than the functional changes, i.e., an experimental animal or a patient may have a poorly functioning liver for some time before actual lesions can be demonstrated. The integrity of the liver—both functionally and structurally—may be jeopardized for some time before it is finally compromised.

From such evidence and speculation it is apparent that the disturbance in the liver is presumably the result of a metabolic injury. We can propose two alternative mechanisms for its genesis. First, this may represent acute nutritional failure. In view of the lack of specific data regarding the dietary habits of these three patients for the period of time shortly preceding and during the early stages of their illness, nutritional failure cannot be excluded. Certainly, one cannot specify which nutrient or combination of nutrients was deficient in relation to the demand for it. Alternatively, the physiologic failure of the liver and its structural changes may conceivably be the result of the action of some humoral agent which either is present because the patient is pregnant or exerts an abnormally destructive effect in the pregnant state. Whether such a hypothetic substance might be a hormone or toxin is, again, a matter for speculation. Support for either hypothesis could be adduced from the case reported by Whitacre and Fang in which rather rapid recovery ensued following intensive therapeutic efforts at supplying the liver with various substances required for the maintenance of its structural as well as its functional integrity, *and* following evacuation of the products of conception. Whether either of these hypotheses is correct, or whether this lesion is the result of another mechanism, it is apparent that the metabolic injury to the liver is one which interrupts the conversion and mobilization of fat, possibly due either to inhibition of an enzyme system or to biologic anatagonism to a necessary metabolite, or both. We would speculate further that the ability of the liver to perform transmethylation is seriously compromised at an early stage in the development of this process. We have no evidence to inform us at which point in its development the liver lesion might become irreversible if, indeed, it ever does. Nor is there any information which would permit one to speculate whether reversal of the morphologic changes would be complete, or whether healing would or would not be accompanied by diffuse fibrosis.

There are certain therapeutic implications which must be stated, however fragmentary our knowledge of this distinctive syndrome may be. Once an antemortem diagnosis has been established—and needle biopsy of the liver seems to be the only present method of diagnosis—vigorous treatment of the patient with fat-free, protein-high intake as well as parenteral fluids and vitamins as indicated seems to be the course of prudence. The administration of choline and methionine has a justification in studies on experimental animals; while it is doubtful that they can be dangerous to human patients, their therapeutic value is still *sub judice*. The use of parenteral protein hydrolysates would appear to be optional. The question of interruption of pregnancy if the patient does not respond promptly to vigorous therapy should be considered. The high fetal mortality in these cases as well as the progressive nature of the disease suggest that, if the diagnosis and therapeutic trial are made promptly, interruption of pregnancy might be in the best interests of mother and child.

In this respect an analogy might be drawn from studies of cases of epidemic infectious hepatitis acquired during pregnancy. Apparently, the usual epidemic mortality of 5 per cent is increased to 20–30 per cent in cases acquired during the last trimester of pregnancy, presumably because of the decreased margin of hepatic reserve. Likewise, in eclampsia, also a disease of the last trimester, in which hepatic lesions are commonly found, the spontaneous or operative evacuation of the products of conception is often of therapeutic benefit. It is debatable whether the formerly designated variety of toxemia also known as acute yellow atrophy was identical with fulminant epidemic hepatitis, or with the distinctive fatty metamorphosis here described, or whether indeed, the term, acute

yellow atrophy, was applied indiscriminately to both lesions because of the difficulty in distinguishing between them clinically or the failure to use precise histopathologic criteria in distinguishing between the two lesions, in patients who received the benefit of autopsy.

SUMMARY

1. Three fatal cases of jaundice occurring at the end of the last trimester of pregnancy are presented. Clinically, the course of these patients is indistinguishable from that of fulminant epidemic (viral) hepatitis. Pathologically, *the lesion is readily distinguishable from infectious hepatitis*, and consists of extensive fatty metamorphosis of liver cells occupying the central two-thirds of the liver lobule. There is no significant infiltration of inflammatory cells nor is there evident necrosis of liver cells. These three cases are similar to those described by Sheehan as "obstetric acute yellow atrophy."

2. The relevant literature is reviewed. Eleven cases have been reported previously. Including the present three cases, only one patient has recovered, a mortality of 92.9 per cent. Presumably, the lesion is reversible.

3. The effect of pregnancy on liver function and its presumptive role in the genesis of this type of lesion is discussed. Likewise, both clinical and experimental methods of protecting the liver from fatty changes are mentioned and possible therapeutic measures are indicated.

4. A variety of experimental data pertaining to fatty changes in the liver is reviewed. These include the role of the liver in inactivation of hormones, the production of fatty livers by dietary deficiencies, by exotoxins, and by biologic antagonism of competitive metabolites (e.g., ethionine). In none of the three clinical cases presented here was there any evidence for or history of dietary deficiency or exposure to or ingestion of an exotoxin. It is inferred that the pathogenesis of this obscure and uncommon lesion lies in some endogenous metabolic aberration which, possibly, produces fatty changes in the liver by interference with enzyme systems, speculatively, those concerned with transmethylation.

5. Chemical analysis of the formalin-fixed liver in one of these cases shows that the increased lipid lies almost entirely in the fatty acid moiety.

6. Fatty metamorphosis was also present in the renal tubular epithelium in all three cases.

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Disturbances of Impulse Formation and Conduction in the Pre-excitation (WPW) Syndrome—Their Bearing on Its Mechanism*

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SOLUTION of the riddle of ventricular pre-excitation (the Wolff-Parkinson-White (WPW) syndrome) has been attempted in various ways. The problem has been studied experimentally and at the necropsy table. Special electrocardiographic leads and vectorcardiography have been used to trace the course of the anomalous pre-excitation (delta) wave. The response of ventricular pre-excitation to extraneous influences like exercise, nerve stimulation and drugs has been recorded in the electrocardiogram and analyzed. Alterations of cardiodynamics associated with the appearance of the anomalous beats were investigated with the help of cardiac catheterization and electrokymography.

Exhaustive and excellent reviews of the voluminous literature appeared about a decade ago[1,2] and recently.[3,4] Among the many theories which have been advanced, differing in fundamental concepts or in shading, two major dissenting views have evolved, both compatible with present knowledge of cardiac physiology; in fact, both were considered in one of the original studies.[5] The first is the hypothesis of an hyperexcitable ventricular focus responding (during sinus rhythm) prematurely to the mechanical or electrical stimulus of atrial activity and leading, on occasion, to a burst of rapid heart action. In its favor has been claimed particularly the possibility of eliciting, in man as well as in the experimental animal, patterns of ventricular pre-excitation by mechanical stimulation of the ventricular septum,[4,6] e.g., in the course of cardiac catheterization,[7,8] and the observation of ventricular premature beats of pre-excitation contour.[9] However, this concept fails to account for the predominance of supraventricular tachycardia in the syndrome, nor can it explain the occurrence of pre-excitation complexes during auricular fibrillation and in association with A-V block. The other concept implies the presence of one (or more) accessory conduction pathways[10–12] bypassing the physiologic impedance to conduction in the A-V node. This view is supported by the anatomic confirmation of such anomalous A-V bridges in man in about half of the cases examined at necropsy,[1,13–19] and the successful experimental reproduction of such a bypass syndrome.[20] Moreover it has the advantage that it can account satisfactorily for the many facets of the syndrome, including the various types of ectopic impulse formation and the occurrence of pre-excitation in the presence of A-V block.

Since in this controversy of views the role of cardiac arrhythmias has received only secondary attention, it seemed worth while to concentrate upon this aspect of the pre-excitation syndrome in the hope that its evaluation in the light of the two conflicting concepts might aid in solution of the problem. The following report deals with five cases of ventricular pre-excitation selected because of some particular features of disturbance of impulse formation or conduction, all, we believe, having a definite bearing on the understanding of mechanisms involved in the pre-excitation syndrome. Figures 1 and 2 show the multiplicity of ectopic supraventricular rhythms which may be observed on different occasions in the same patient. In addition, Figure 1 illustrates the various types of ventricular response to rapid impulse formation, and Figure 2 the different responses to carotid sinus stimulation.

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In Figure 3 is reproduced an unusual case of ventricular pre-excitation in the presence of an abnormally prolonged A-V conduction time. Figure 4 illustrates the operation of an independent parasystolic focus in pre-excitation, while in Figure 5 an ectopic focus, apparently located within an anomalous A-V connection, acts to cause single and regular runs of ectopic beats. For a detailed analysis of each case, and an evaluation with respect to the two pre-excitation theories, the reader is referred to the section on Illustrative Appendix. Our viewpoints on the mechanism of ventricular pre-excitation, arrived at on the basis of the interpretation of these five cases, of additional material available in the files of the Heart Station of the Michael Reese Hospital, and a study of the pertinent literature, are outlined subsequently. New evidence was found in the course of this study to fortify the hypothesis that an anomalous A-V connection is in operation in the pre-excitation syndrome.

The Pre-excitation Beat. The typical electrocardiographic contour of the pre-excitation complex has the characteristics of a ventricular fusion beat.[1,21,22] Its early anomalous component,[23] the delta wave,[24] indicates that in some way activation of the ventricles starts in a devious fashion, by early excitation of certain portions of the myocardium, ahead of the impulse travelling down the A-V junction over normal pathways. The latter may or may not participate in ventricular activation. If it does, a slender terminal part of the QRS deflection interrupts, or is added to, the delta wave. Unlike the ordinary type of fusion beats which is caused by ventricular interference of two different impulses, one usually originating in the atria and the other in the ventricles, in pre-excitation it is the sinus impulse which splits somewhere on its way toward the ventricles and one (or more) of its offspring takes an anomalous route in reaching them. Pre-excitation results because at least one of the paths which the split sinus impulse uses is a rapid one which avoids physiologic obstacles to impulse conduction through the A-V node. It is for this reason that in pre-excitation the anomalous component invariably forms the initial portion of the resulting fusion beat. On the other hand, like the ordinary ventricular fusion beat, the pre-excitation complex may vary in contour from beat to beat and from time to time, and cause more or less abnormality in the duration and contour of QRS, and in the shape of ST-T. This is clearly brought out in Figure 1. It obviously will also vary from case to case. The similarity of fusion beats caused by ectopic impulses and those caused by pre-excitation may be very close.[1] In fact, the former have been mistaken for the latter even in investigations dealing with the mechanism of pre-excitation.[25]

Apart from the varying degrees of asynchronism of the two fractions of the sinus impulse in the ventricles, other factors may also influence the appearance of pre-excitation beats from case to case, viz., the position of the heart, the alteration of the fundamental electrocardiographic contour by ventricular strain or myocardial infarction, and the location of the point at which the anomalous impulse enters the ventricles. The classic combination of a short P-R and a wide QRS need not always be present to arrive at the diagnosis of a pre-excitation (WPW) syndrome, but then the differentiation from other conditions may not always be possible. Thus records occur with upright P waves, abnormally short P-R intervals, but with a QRS of normal duration which may be interpreted as coronary nodal rhythm[5,31]* as well as pre-excitation beats,[26,27] particularly when attacks of rapid heart action occur in such persons. On the other hand, unquestionable pre-excitation beats may occur with a normal P-R interval[23] or, as is demonstrated clearly in Figure 3, even with an abnormally prolonged one. Persistent ventricular bigeminy, with bizarre beats occurring in every other sinus cycle, just after the sinus P wave, may be caused by ventricular pre-excitation in alternate beats or by late diastolic ventricular ectopic beats. The differentiation of the two is impossible in a single record.[9] In general, the diagnosis of pre-excitation should be made only when respective beats terminate at least two successive cycles, and the P-R of these beats does not change, regardless of its actual length. (Figure 3.) If it does, ventricular fusion beats caused by ectopic impulses, at a rate approaching that of the sinus pacemaker, are more likely.[4,6,8,25,28]

Cardiac Arrhythmias in the Pre-excitation Syndrome. The most frequent disturbance of rhythm, encountered in about three-fourths of all cases,[23] is a supraventricular type of paroxysmal tachycardia with regular spacing and participation of the atria in the rapid ventricular beating. It is, however, not always possible to establish the relationship of the P waves to the QRST preceding or following them and hence to

* Figure 3II of this study.

distinguish between an auricular or nodal type of tachycardia. Next in frequency are auricular fibrillation and flutter, particularly when pre-excitation is present in rheumatic heart disease. Sometimes all three types of auricular arrhythmias can be seen on different occasions in the same patient, as in Figure 2. Ventricular paroxysmal tachycardia appears to be extremely rare. We have observed no case which unequivocally falls into this category. Most of the reported cases are open to an alternative interpretation, the most common being auricular fibrillation with persistent or intermittent ventricular pre-excitation.[17] This problem is dealt with in Figure 1C.

Isolated ectopic premature systoles occur in the pre-excitation syndrome as elsewhere and may be auricular, nodal or ventricular in origin. An unusual instance of ventricular parasystole unrelated to the syndrome is demonstrated in Figure 4. "Passive" ectopic impulse formation (nodal escape and A-V dissociation) can be observed in the pre-excitation syndrome. It is usually elicited by carotid sinus pressure or other types of vagal stimulation applied in order to break a paroxysm of tachycardia or for informative purposes. An example of the latter is shown in Figure 1B. Ectopic nodal beats, premature or escaped, ordinarily show no pre-excitation component[3,11,22,26,29,30] but on occasion they may.[11,24,31]

Finally, rare cases are on record in which ventricular pre-excitation occurred in the presence of second degree A-V block.[12,15,29,32] A unique instance associated with first degree A-V block is reproduced in Figure 3.

The frequency of certain types of cardiac arrhythmias in association with ventricular pre-excitation indicates that they are an integral part of the entire syndrome. Any attempt to explain the syndrome and any concept advanced must account for the occurrence of the phenomenon of pre-excitation under the various circumstances mentioned, and should explain the concomitant disorders of formation and conduction of the cardiac impulse on the basis of physiologically acceptable principles. Careful consideration of the pros and cons of the two main theories as to their applicability to simple and complex arrhythmias leads us to the conclusion that the hypothesis of an accessory A-V connection is most appropriate to account for all of the known aspects of the pre-excitation syndrome.

We see, therefore, no present need for implication of any other theory so far advanced.

The Properties of the Anomalous A-V Connection and Their Bearing Upon the Manifestations of the Pre-excitation Syndrome. The accessory atrioventricular bridge appears to be a congenital anomaly since pre-excitation occurs in the newborn and even in the premature,[33] and sometimes in association with other cardiac malformations,[14,19,34] and is possibly a hereditary anomaly since the syndrome has been found in several members of one family.[1,35] It may represent remnants of the embryonic atrioventricular canal wall dislocated during its later incorporation into the adult A-V junction and its differentiation into specific muscle tissue.[5,11] Such anomalous muscle fibres may be remote from the A-V node (eccentric)[1,13,15] or may connect the latter and the common A-V bundle as a short cut to the upper portions of the ventricular septum.[14] Depending on the number, the location and the length of such accessory muscular A-V connections the electrocardiographic pattern of ventricular pre-excitation and the associated manifestations will vary from case to case. Thus an "eccentric" muscle bridge will tend to produce a pronounced delta wave (as seen for instance in Figure 2), or pre-excitation may dominate the ventricular activation so as to minimize the excitation over normal pathways.[36] Conversely, a short anomalous bridge running close to the A-V node and quickly uniting with normal A-V pathways may cause pre-excitation (manifested by a shortened P-R) and yet not alter the mode and duration of ventricular activation significantly.[26,27] An origin of the accessory A-V connection within the A-V node is suggested when ectopic (nodal) beats and beats of sinus origin show an identical pattern of pre-excitation;[21,31] or when pre-excitation is associated with A-V block. (Figure 3.) There may be more than one accessory pathway;[11,13] one may cause pre-excitation, the other may be used preferentially by impulses originating in the A-V node.[37] This could explain the multiplicity of phenomena in our Figure 2.

The property of conductivity in the anomalous A-V bypass may appear early in life and persist over many years[38] or it may be latent at first and become manifest later,[39] spontaneously or when normal conduction pathways are diseased ("acquired WPW syndrome").[1,12,16] Rapid conduction through such fibres may be persistent or intermittent and may be unidirectional or bi-

directional. The fact that in the ectopic anomalous beats of Figure 5 retrograde activation of the atria precedes that of the ventricles can be explained on this basis. Variations in conductivity in the accessory pathway under the influence of nerves and drugs do not always parallel those of the normal pathway.[23] Sometimes only the anomalous path is affected, sometimes only the normal one and sometimes both pathways. Under the last circumstances the effects may be quantitatively different though in the same direction. Parasympathomimetic drugs and digitalis appear to affect adversely only normal A-V conduction, whereas quinidine and pronestyl® depress conductivity through the bypass.[17,29,40,41,42] Such drug effects can be used to verify the presence of pre-excitation in doubtful cases or to unmask other unrelated electrocardiographic alterations.[43] An unusual example of independent changes of impulse transmission in the two pathways achieved by carotid sinus stimulation is shown in Figure 2B.

Independent variations of refractoriness and recovery in the two pathways may influence the pattern of pre-excitation especially during rapid stimulation by auricular premature systoles (Fig. 1B) or in auricular fibrillation. (Fig. 1C.) Such inequality in the conditions for impulse transmission may lead to incomplete penetration of one of the pathways. Retrograde penetration of one pathway by impulses which have passed down the other can occur in this way, and may prevent subsequent stimulation of the former from the atrial end. This mechanism —a variety of concealed conduction—may account for temporary disappearance, or appearance, of pre-excitation during rapid heart action, as exemplified in Figure 1C. Common types of aberrant ventricular conduction are seen especially at the onset of paroxysmal tachycardia (Fig. 1B) and may complicate interpretation of such records.[17]

Since an accessory A-V conduction path may have all these properties it is possible to explain satisfactorily all varieties of rapid heart action associated with pre-excitation. Impulses conducted in a normal fashion through the A-V junction to the ventricles may return back toward the atria via the anomalous path[10] (a variety of re-entry), and continuation of such a re-entry mechanism may initiate and perpetuate rapid heart action. In support of such a view is the fact that in cases in which the onset of supraventricular tachycardia was recorded the last beat preceding the tachycardia was not of the pre-excitation type.[11,44–47] (This is also the case in Figure 1B.) It is our belief that when the path of the circulating wave thus initiated includes a large portion of the atrial myocardium, the atria will take part in the paroxysmal tachycardia. The P wave during this tachycardia may be upright or retrograde. It will be upright when during the atrioventricular reciprocation the circulating wave enters the atria in a region which is remote from the A-V node, and at such a point as to cause atrial activation to take place in a direction similar to normal, that is, from right to left. A retrograde P wave may result either when the circulating wave enters the atria from the left, remotely from both A-V and S-A nodes, under which circumstances the atrial invasion will precede re-entry into the A-V node; or when the anomalous path originates close to or within the A-V node, under which circumstances the atria may not be included in the re-entry path but will be activated in retrograde fashion by impulses radiating from the re-entry sweep. A "nodal" type of paroxysmal tachycardia will then be the result. It is conceivable that in the latter case the atria remain under the control of the sinus node, with A-V dissociation, but this appears to be extremely rare. Under all these circumstances atrial activation takes place in a coordinated manner. However, should the first or one of the later impulses returning to the atria via the anomalous path reach the atria very early in their cycle, while they are partially refractory and in their "vulnerable phase," it may initiate auricular fibrillation[3] (or flutter). It is possible that paroxysmal auricular tachycardias preceding the onset of flutter and fibrillation, as seen in Figure 2, are initiated in this way, and not by way of the re-entry mechanism described.

With the onset of auricular fibrillation (or flutter), A-V reciprocation is stopped and impulses of the fibrillating atria will start to pass down in rapid irregular sequence either over the normal or the anomalous path, depending on their state of recovery from preceding stimulation. Since each impulse passing down one path has a tendency to penetrate in retrograde fashion into the other, thus rendering it refractory,[17] normal or anomalous, activation of the ventricles will continue for some time until, during a longer ventricular pause, the other pathway recovers and becomes dominant. (Fig. 1C.) Due to this mechanism, the anomalous

beats during auricular fibrillation are usually wider than during sinus rhythm, being entirely composed of the delta wave—the ordinary fusion beats of pre-excitation occur only at the time of transition of one type of ventricular excitation into the other.[17]

The occurrence of pre-excitation beats during auricular fibrillation, in which there is no synergic auricular contraction, is a strong argument against the concept that in sinus rhythm with pre-excitation auricular contraction by mechanical stimulation causes premature discharge of an irritable ventricular focus and that the impulse so generated interferes with the sinus impulse travelling only over normal A-V paths. It favors the view that an anomalous path must be involved. Another strong point favoring the concept of anomalous A-V conduction, and opposed to the concept of an irritable ventricular focus, is the occurrence of pre-excitation complexes in cases with A-V block. In instances of second degree A-V block[12,15,29,32] with dropped ventricular beats it is hard to conceive how failure of response of the supposed ventricular focus occurs simultaneously with failure of the sinus impulse to pass the A-V junction. In the instance of first degree A-V block illustrated in Figure 3 latency of response of the ectopic focus synchronized with the delay of A-V conduction would have to be postulated, a highly improbable circumstance. Implication of an A-V conduction disturbance affecting two conduction pathways, one of which is anomalous, offers a much more reasonable and simple explanation. It is possible that the impulse might be delayed or stopped simultaneously in both pathways. (Fig. 1B.) When, however, the anomalous path is assumed to originate in or below the A-V node, only one area of block, within the node, need be postulated.

On rare occasions ectopic beats have been observed in this syndrome which partly or entirely match the contour of the sinus beats with pre-excitation but occur unrelated to a P wave,[9,29] are linked at a shortened P-R to an anomalous P[9,48] or are preceded by a frankly retrograde P at an abnormally short P-R.[49,50] An example of the latter is shown in Figure 5. Such beats, it would appear to us, originate in the anomalous path itself.

The recognition that impulse formation may on rare occasions take place in the anomalous fibres does not contradict the concept that the principal role of the accessory path is to accelerate A-V conduction and thus to produce the syndrome of pre-excitation. As in ordinary A-V junctional tissue, two properties may exist in a congenitally displaced portion of it. It appears that the property of conductivity of such fibres, both in a forward and in a retrograde direction, is the primary factor responsible for the usual spontaneous manifestations of the syndrome, and that the property of rhythmicity ordinarily is absent or dormant. Rarely, the latter too may come into operation, and then causes isolated premature systoles, or takes over the pacemaker function of the heart, as occurred in the case illustrated in Figure 5.

It has been stated that "delayed anomalous conduction which would furnish the final link in the chain of evidence for a functioning structural accessory (A-V) pathway has never been observed;"[23] and that "there is no proof that stimuli originate in the anomalous pathway."[12] We believe that these two missing links are provided in the two observations reproduced in Figures 3 and 5. Future similar demonstrations of such a dual function of the supposed anomalous fibres should prove the case in favor of the presence of an accessory A-V connection in the pre-excitation syndrome.

SUMMARY AND CONCLUSIONS

1. Five selected cases of the pre-excitation (WPW) syndrome which exhibited various types of disturbance of formation and conduction of the cardiac impulse were submitted to detailed analysis and examined as to the applicability of the two fundamental concepts of pre-excitation, the hypothesis of an hyperexcitable ventricular focus and that of an anomalous A-V conduction bypass.

2. In all five instances the hypothesis of one or more anomalous A-V connections proved most appropriate to account for all aspects of the pre-excitation syndrome, including various simple and complex arrhythmias. This was true even in the presence of a ventricular parasystolic center.

3. In one case in which ventricular pre-excitation occurred in association with an abnormally prolonged P-R interval, the accessory A-V junction was considered to originate in the lower portion of, or below, the A-V node. In another instance, intermittent spontaneous impulse formation in an accessory A-V bridge had to be postulated to account for all characteristics of the ectopic beats occurring as

sporadic premature systoles or in the form of a paroxysmal tachycardia. Delayed anomalous excitation, and impulse formation in the anomalous bypass, demonstrable in these two cases, represent two missing links postulated as additional evidence for the concept of an accessory A-V connection.

4. On this basis it would appear that the anomalous muscular A-V connection present in patients with the pre-excitation syndrome has the special properties ordinarily attributed to specific cardiac tissue, viz., conductivity and rhythmicity. The property of conductivity predominates and is the one which is responsible for the common spontaneous manifestations of ventricular pre-excitation, including the various associated disorders of rhythm. The property of rhythmicity, when present, ordinarily is dormant. In exceptional instances it may become manifest and lead to isolated ectopic beats or to ectopic rhythms.

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ADDENDUM

Since this study was submitted for publication further proof of the existence of accessory A-V connections was provided by Lev, Gibson and Miller (*Am. Heart J.*, 49: 725, 1955) by careful histologic study of the entire conduction system and both A-V rings in a case of Ebstein's disease associated with ventricular pre-excitation. About the same time, Borduas, Rakita, Kennamer and Prinzmetal (*Circulation*, 11: 69, 1955) advanced the view that the Wolff-Parkinson-White syndrome is an example of "accelerated A-V conduction," a hitherto unrecognized abnormal mechanism operating within the A-V node. As evidence for this concept they presented short strips of clinical and experimental electrocardiograms which, in our judgment, may be interpreted as: (1) nodal escape and nodal rhythm leading to intermittent A-V dissociation, (2) ventricular premature systoles interfering with A-V conduction of sinus impulses, or (3) 2:1 A-V block in which the P-R duration exceeds the P-P interval.

ILLUSTRATIVE APPENDIX

Figure 1. All strips are lead I recorded on different occasions in a sixty year old man with hypertension.

In strip A, during sinus rhythm (rate 64), the pre-excitation syndrome is revealed by the combination of a short P-R (0.08 sec.) and a small delta wave widening QRS to 0.10 sec. Four such sinus beats are seen in strip B viz., the first two and the fifth and seventh beats (S-T is depressed, unlike those in A). Each of these beats except the first is followed by two premature P waves (pairs of auricular premature systoles) giving rise to an upright notch in the ST-T of the preceding beat. The first of these pairs of premature P waves is conducted and leads to a pre-excitation beat with an exaggerated delta wave and a negative ST-T. In these beats a greater amount of ventricular muscle is stimulated in an anomalous fashion than in the sinus beats. The second P wave of the pair occurs at a shorter P-P interval than the first. In the second group it is non-conducted; in the first and third groups it is conducted, giving rise to a totally different diphasic ventricular complex, with a tall upright T wave and a QRS of 0.10 sec. but without a delta wave. In these beats ventricular activation takes place entirely over ordinary A-V conduction pathways but this is altered by aberrant ventricular conduction such as occurs commonly in early auricular premature systoles. In the third group, this beat, in turn, is followed by a run of regularly spaced beats (rate of 188) of normal QRS duration (0.07 sec.) with a P wave preceding each ventricular complex (auricular paroxysmal tachycardia).

The variety of ventricular complexes encountered in strip B and their recurrent grouping can be explained appropriately by assuming the presence of two A-V conduction pathways having independent characteristics of refractoriness and recovery. When the rate is slow (as in A), conduction over the ordinary pathways dominates over that through the anomalous one, resulting in the appearance of only a small delta wave. This dominance of the ordinary path lessens in the first of the premature beats (in B), apparently because recovery is faster in the bypass. However, in the second of the pair of conducted premature beats the dominance is reversed in favor of normal A-V conduction. This paradoxic behavior of the two pathways cannot be accounted for by simple recovery phenomena. It may be that the cycle length preceding the beat ahead of a premature impulse exerts a more pronounced influence upon the duration of the refractory period of the

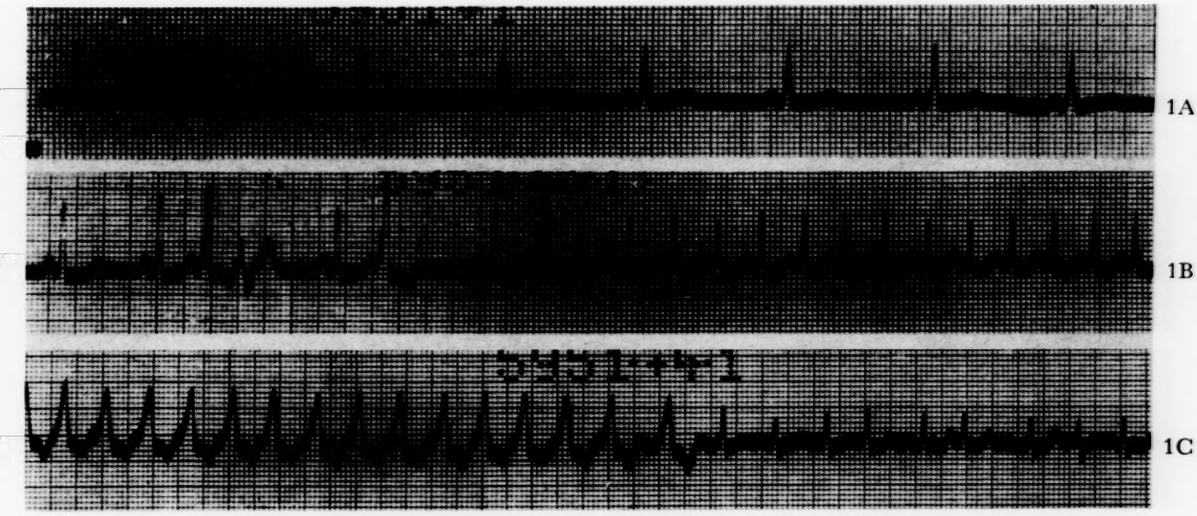


FIG. 1. A case of pre-excitation (WPW) syndrome (A) associated with auricular premature beats and a paroxysm of supraventricular tachycardia (B) and a paroxysm of auricular fibrillation (C). (See text.)

ordinary path than of the anomalous one, so that after two successive short cycles conduction is enhanced over the normal A-V conduction pathways.

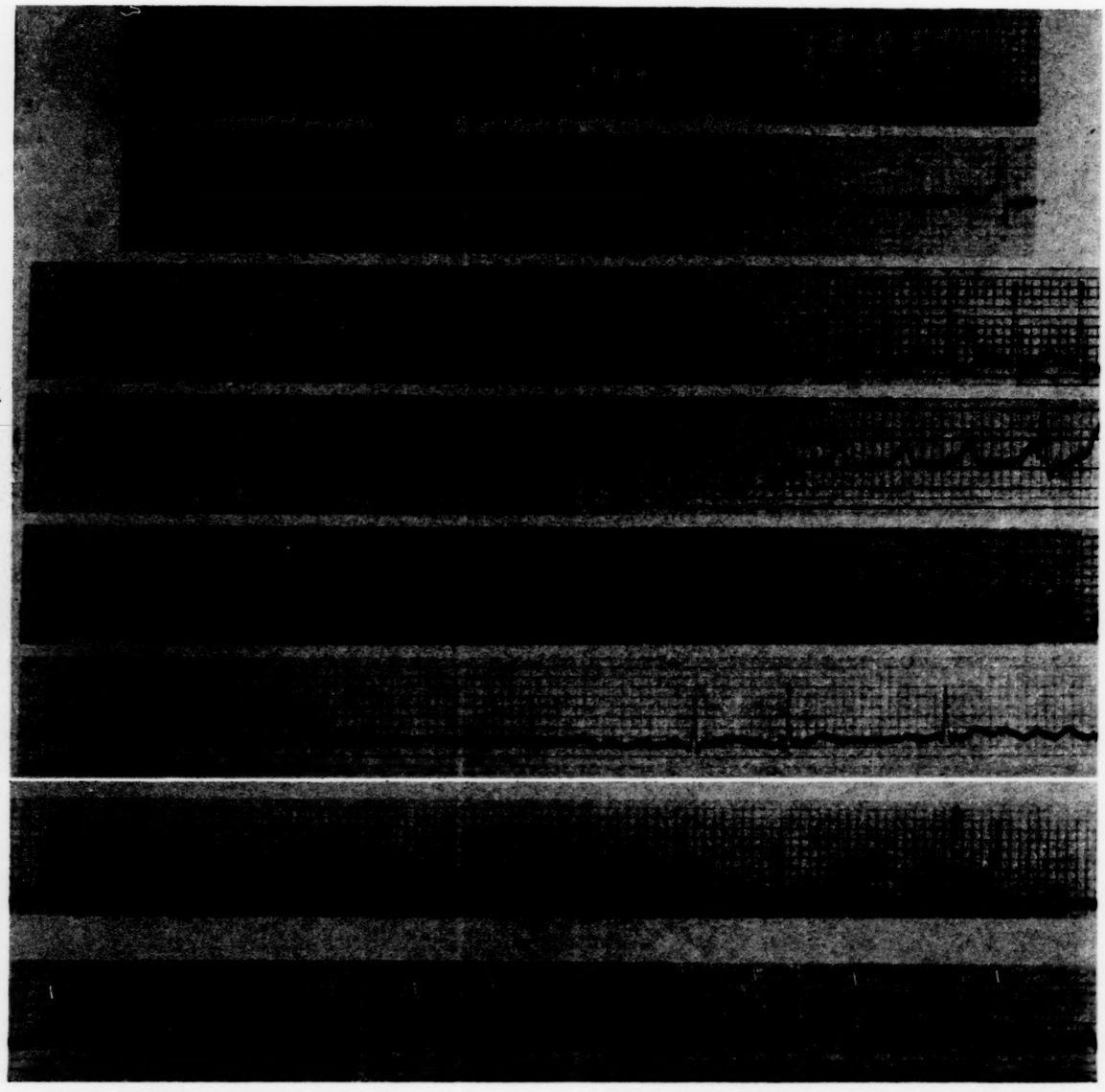
The second impulse of the pairs of premature systoles occurs so early that it leads to the usual type of aberrant ventricular conduction and, on one occasion, does not yield a ventricular response. In the latter case it must have been stopped in both pathways.

The absence of the delta wave during the stabilized period of rapid heart action at the end of strip B shows that only normal A-V conduction pathways are used in transmitting the impulse from the atria to the ventricles. Apparently the second premature impulse in the last group of premature beats, after activating the ventricles, succeeded in penetrating the bypass backward to reach and reactivate the atria, and thus initiated continued atrioventricular reciprocation. Obviously because the bypass was used for retrograde conduction it could not be entered by the rapid auricular impulses.

In strip C the diagnosis of auricular fibrillation is made on the basis of the irregular rapid ventricular beats and the presence of irregular undulations (f waves) clearly seen in the second half of the strip. The run of bizarre unevenly spaced ventricular complexes in the first half of the record is not due to ventricular paroxysmal tachycardia (which it resembles) but to conduction of auricular impulses through the anomalous bypass. This is established by comparing these beats with the pre-excitation beats in A and B. At first, during auricular fibrillation, the delta wave occupies the entire upstroke and QRS measures 0.14 second, suggesting that the ventricular activation is effected entirely by the anomalous path. Apparently, impulses constantly are penetrating into the normal A-V conduction pathways from the ventricular end and render the A-V node refractory to impulses from the fibrillating atria (a variety of concealed conduction). However, in the middle of the record, when the ventricular rate transiently slows, one of the auricular impulses succeeds in completely traversing the normal A-V pathways in a forward direction and immediately conditions are reversed. This impulse after reaching the ventricles then penetrates into the bypass in retrograde fashion and so renders this path refractory to auricular impulses. This continues and the result is persistent normal forward A-V conduction up to the end of the record. The slight variations in the QRST contour during this latter period is a common occurrence in ordinary auricular fibrillation.

In summary, this case illustrates: (1) the variety of types of ectopic auricular impulse formation which may accompany the pre-excitation syndrome; (2) the variations in QRST contour caused by the availability of an accessory conduction pathway; and (3) the problem of differentiating ventricular paroxysmal tachycardia from the combination of pre-excitation with auricular fibrillation.

The fact that ventricular pre-excitation occurs during bouts of auricular fibrillation excludes



2A

2B

FIG. 2A and B. Ectopic supraventricular rhythms (A) and effects of carotid sinus pressure (B) in the pre-excitation (WPW) syndrome. (See text.)

the view that the anomalous component of QRS is due to mechanical stimulation by the atrial contraction of an irritable focus in the ventricles, since such a synergic contraction of the atria is not present in auricular fibrillation. On the other hand, it is no more difficult to explain the occurrence of auricular fibrillation (and flutter) in the pre-excitation syndrome by the assumption of an accessory A-V pathway than that of other forms of paroxysmal supraventricular tachycardia. If a single auricular impulse, having traversed the normal A-V conduction pathway, returns to the atria via the anomalous bypass and reaches the atria early, in the "vulnerable period," auricular fibrillation (or flutter) may be initiated instead of a reciprocating mechanism between atria and ventricles which causes regular supraventricular paroxysmal tachycardia.

Thus the assumption of an anomalous A-V bypass can best account for all varieties of disturbance of rhythm and alterations in QRST contour encountered in this case.

Figure 2. The records shown (all in lead II, with time lines indicating 0.05 sec.) are portions of numerous tracings obtained in a twenty-year old man with rheumatic heart disease.

The variety of A-V conduction patterns ob-

served during periods of different rhythm are shown in A. In the top strip there is sinus bradycardia (rate 50) with normal A-V (P-R, 0.20 sec.) and intraventricular (QRS, 0.08 sec.) conduction. In the second strip in which the sinus rate varies between 57 and 67, the last three beats reveal a contour characteristic for ventricular pre-excitation in that QRS is prolonged to 0.14 sec. by a distinct delta wave. The latter starts immediately after a small terminal notch of the P wave so that P-R is shortened to 0.12 sec. The demarcation of the anomalous from the normal component of ventricular activation is very sharp. The first two beats of this strip differ in contour from both the normal (in the top strip) and the pre-excitation beats. Their QRS is 0.11 sec. and their P-R distance varies. These are ectopic (nodal) beats, activating the ventricles during a period of A-V dissociation. The reasons for the slight aberration of their contour is discussed later. The lower four strips exemplify various types of disturbances of atrial rhythm observed in this patient. In the sequence from above down, there is, first, a paroxysmal auricular tachycardia (rate 172) with 1:1 A-V conduction. In the next strip the same tachycardia is seen at a slightly faster rate (180) with irregular A-V conduction varying between 3:1 and 5:1 ratios. In the next strip there is auricular flutter (rate 300) with regular 4:1 A-V conduction, and in the last strip auricular fibrillation has developed (rate about 400) with a slow irregular ventricular response (average 60). All these varieties of rapid auricular action cause ventricular activation via normal A-V conduction pathways as evidenced by the normal contour and duration (0.08 sec.) of QRS; (compare with top strip).

Various effects of carotid sinus pressure upon A-V conduction and ventricular activation during sinus rhythm are shown in B. In the top strip, which starts with a pre-excitation beat, carotid sinus stimulation (↓) causes slowing of the sinus rate. The node escapes and causes, by A-V interference, a period of A-V dissociation until, after release of carotid sinus pressure (↑), the ventricular pre-excitation by the sinus impulse is resumed. The nodal beats show the same slight aberrant contour seen at the beginning of the second strip of A. In the lower strip, the effect of carotid sinus pressure during the pre-excitation is more marked and causes a period of complete standstill of 3.2-second duration because both sinus and A-V nodes are suppressed. The first beat after the onset of the carotid sinus pressure (↓), and the three beats following the pause, differ in contour from the pre-excitation complexes ordinarily seen in this patient in that the amplitude of the terminal narrow portion is smaller, whereas the delta wave is somewhat taller.

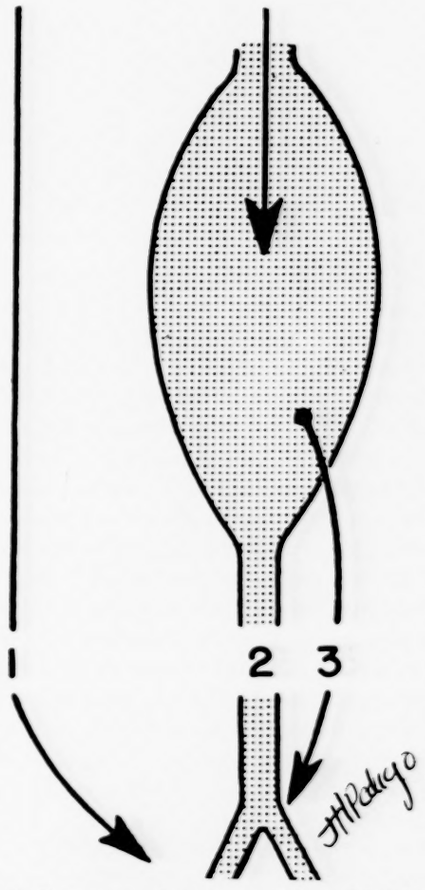


FIG. 2C. A diagrammatic representation of the three A-V conduction pathways assumed to be present in Figures 2A and 2B.

The observations in this patient's records can be summarized as follows: Four types of ventricular beats are present, viz., (1) the normal type seen with both sinus and ectopic rapid auricular rhythms; (2) ordinary pre-excitation beats seen intermittently only during sinus rhythm; (3) modified pre-excitation beats occurring with carotid sinus stimulation, and (4) ectopic supraventricular beats, nodal in origin, with aberrant ventricular contour. Carotid sinus stimulation, besides altering the contour of the pre-excitation beats, caused sinus slowing with nodal escape, or simultaneous standstill of both the sinus and A-V nodes.

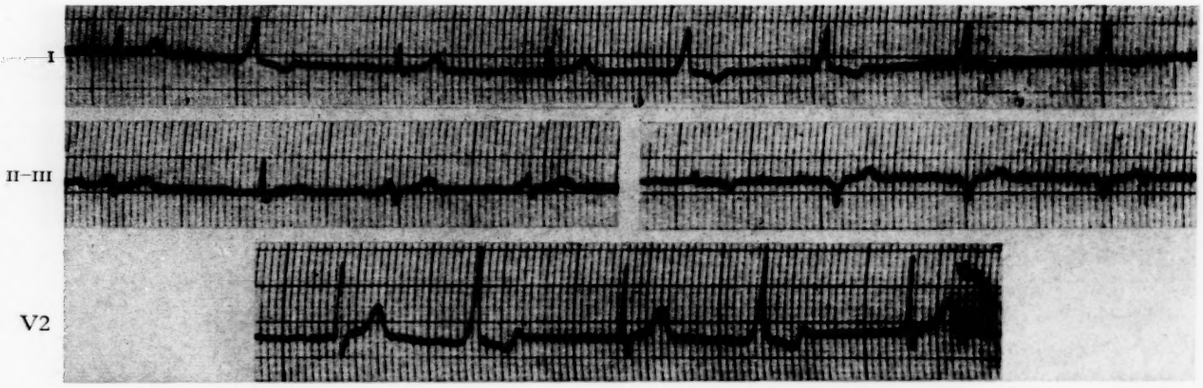


FIG. 3. Pre-excitation (WPW) syndrome with prolonged P-R interval in a thirty-two year old man, asymptomatic and without clinical evidence of heart disease. (Time lines 0.05 sec.) (See text.)

While it seems impossible to account for this combination of phenomena on a uniform basis by implicating an hyperexcitable ventricular focus, they can be explained on the assumption that there are three A-V conduction pathways, as indicated in the accompanying diagram 2C. In this diagram (1) represents an anomalous pathway bypassing the A-V node, (2) represents the ordinary normal conduction pathway, and (3) represents a preferential path near (2) used, as is often the case, by impulses of nodal origin. The sinus impulses, in stimulating the ventricles, use either path (2)—with a normal QRS resulting; or both (1) and (2)—with a pre-excitation beat resulting. Nodal impulses stimulate the ventricles over their preferential pathway (3) whereas the ectopic auricular impulses (during auricular tachycardia, flutter and fibrillation) can use pathway (2) only. Pathway (1) is refractory during the rapid auricular activity because it is persistently penetrated in a backward direction from its ventricular end by the rapid auricular impulses returning to the atria, a re-entry mechanism which may be responsible for initiation as well as perpetuation of the rapid atrial activity. At times the A-V node escapes because the sinus rate slows below the inherent rate of the node. As a consequence, the forward impulse transmitted from the node over pathway (3) dominates the ventricles. The retrograde nodal impulse interferes with the oncoming sinus impulse and causes A-V dissociation. The absence in nodal beats of a delta wave (indicating ventricular stimulation by an impulse traversing pathway (1)) means that the nodal and sinus impulse interfere with each other either above, within or at the ventricular end of pathway (1). Either of the two impulses (the forward or retrograde one) may penetrate the anomalous pathway, without traversing it, and this would represent a variety of concealed A-V conduction.

The effect of carotid sinus stimulation upon the contour of the pre-excitation beats is the result of depression of the conductivity of pathway (2), so that the sinus impulse which divides to use both pathway (1) and pathway (2) travels more slowly over the latter and, as a consequence, more of the ventricles will be dominated by the impulse reaching it over pathway (1). Therefore, the resulting ventricular fusion beats will show a greater delta and a smaller normal part in the QRS complex.

Again, the hypothesis of anomalous A-V bridges appears to be the only one which can satisfactorily explain all the facts observed, i.e., the variable contour of ventricular beats under different conditions as well as the multiplicity of abnormal rhythms encountered.

Figure 3. In each lead two types of ventricular complexes are seen, one of which is normal whereas the other has a contour typical for ventricular pre-excitation with QRS prolonged to 0.12 sec. The P-R interval of both is abnormally prolonged, although to a lesser degree (0.24 sec.) in the pre-excitation beats than in the beats with normal ventricular activation (0.30 sec.). The interval from the onset of P to the end of QRS is the same in both (0.36 sec.). While in V2 the pre-excitation syndrome could be questioned, and late diastolic ectopic ventricular beats with A-V interference might be suspected, the presence of pre-excitation is established in the three standard limb leads in which anomalous beats at a constant P-R occur several times in succession.

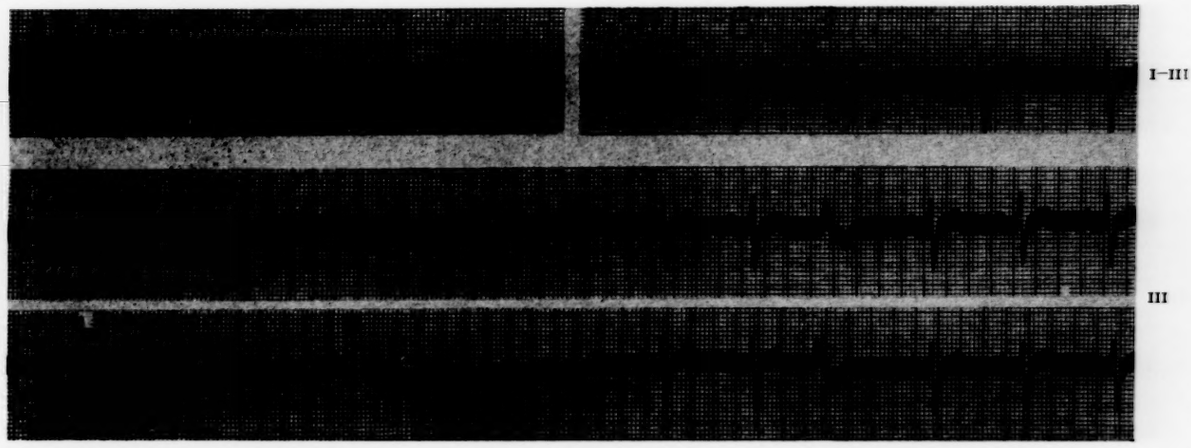


FIG. 4. Ventricular parasystole in the pre-excitation (WPW) syndrome in a seventy-two year old man with chronic coronary insufficiency.

This case is unique because it illustrates that ventricular pre-excitation may take place with a P-R interval still abnormally long. Thus an abnormally short P-R is not a necessary part of the syndrome. Moreover, this observation necessarily leads to certain conclusions, in this particular case and in general, as to the mechanism of ventricular pre-excitation. With such a long P-R interval of the pre-excitation beats it is very difficult to conceive any type of stimulation —mechanical or electrical—of an irritable ventricular focus by atrial activity occurring ahead of the impulse traveling down over normal A-V conduction pathways. Thus one of the two principal concepts advanced seems to be ruled out. If, on the other hand, an anomalous connection is present between atria and ventricles, the block causing the prolonged P-R in both normal and pre-excitation beats must be located above its origin unless simultaneous depression of about equal degree is assumed in both the normal and anomalous pathways; this last is extremely unlikely. Since abnormal impedance to A-V conduction is most commonly located in A-V junctional tissue, and particularly in the A-V node, it can be concluded that the anomalous path, over which the impulse bypasses part of the obstacle of the A-V junction, arises either in the lower portion of the A-V node or below it.

This case may therefore be considered to be a strong link in the chain of evidence in support of the concept ascribing ventricular pre-excitation to an anomalous A-V bridge bypassing the obstacle to impulse conduction in the A-V node, entirely or in part.

Figure 4. The two leads shown in the upper row have typical earmarks of ventricular pre-excitation: upright P waves (rate 59) linked by a constant abnormally short (0.10 sec.) P-R interval to abnormal QRS complexes widened to 0.12 sec. by a delta wave. The last beat in lead I and the third beat in lead III occur prematurely and have a contour totally different from the dominant pre-excitation beats. Their coupling to sinus beats differs and so does their relation to P. In lead III a sinus P wave, in lead I a premature P, can be identified within their S-T. On this basis the diagnosis of ventricular premature systoles is made, causing A-V interference (in III), or retrograde conduction to the atria (in I), depending on the length of their coupling.

Because of the variability of the coupling of the ectopic beats a long record of lead III was obtained on the next day, two continuous portions of which are shown in the two lower strips. (The last beat of the middle strip is reproduced as the first beat of the lowest strip.) The dominant sinus beats (rate 79) and most of the ectopic beats resemble those seen previously in lead III. However, two beats (the fifth in each strip), which follow a sinus P wave, have a contour intermediate between the two types. Both start with a delta wave but in the former the terminal portion of QRS resembles the sinus beats while in the latter it resembles the ectopic beats. These are ventricular fusion beats with ventricular interference between a sinus impulse and an ectopic impulse occurring subsequent to the ventricular pre-excitation. When the intervals between ectopic beats, and ectopic

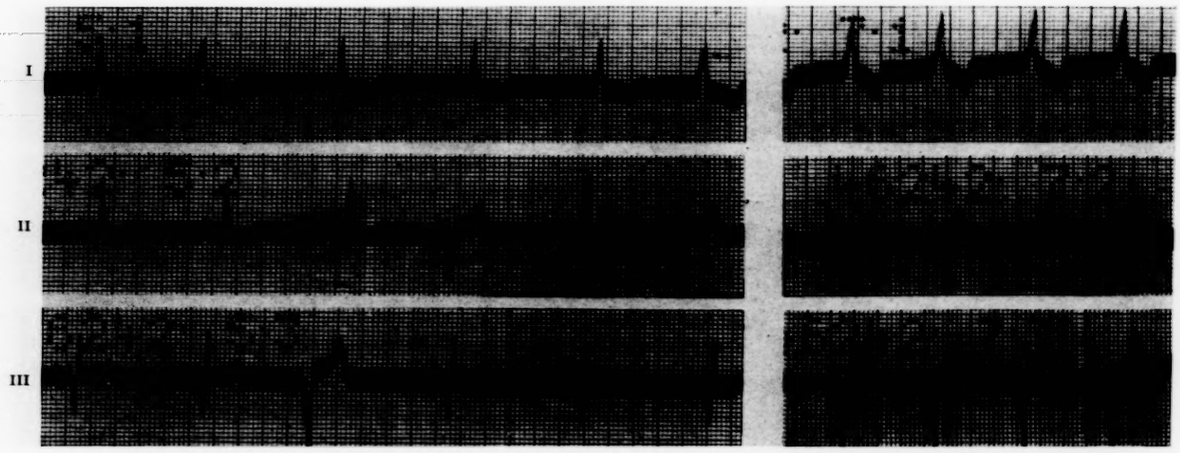


FIG. 5. Pre-excitation (WPW) syndrome with ectopic beats apparently arising in the anomalous path, in a sixty-seven year old man with chronic bronchopulmonary disease. (See text.)

and fusion beats, were measured in the entire record the presence of a ventricular parasystolic focus, discharging at a regular rate of 31, could be established.

An irritable ventricular focus responding with premature impulse formation to the atrial contraction has been invoked to account for the phenomenon of ventricular pre-excitation. Since in this case the autonomous "protected" rhythmic character of the ectopic ventricular focus was unquestionably demonstrated, it cannot be involved in producing the ventricular pre-excitation of the sinus beats. The protection from other impulses which permits maintenance of its steady rhythm renders such an implication impossible. Thus premature ventricular systoles can occur coincidentally with the pre-excitation syndrome, as in any other condition.

Figure 5. In A, in the dominant sinus rhythm (rate 60), the presence of the pre-excitation syndrome is diagnosed on the basis of a short P-R (0.10 sec.) and the presence of a delta wave. The anomalous component, widening QRS to 0.12 sec., starts as an upright deflection in all three leads, largest in I. In addition, there are premature systoles in each lead preceded by premature P waves of retrograde contour, with a P-R less than that of the sinus beats. These beats start with a deflection similar to the delta wave of the sinus beats but unlike the latter, its course is not interrupted by the slender normal positive component of simultaneous ventricular invasion via normal A-V pathways. Thus the entire downward deflection of QRS of the premature beats in leads II and III seems to be caused by the impulse entering the ventricles anomalously.

In B, obtained in the same patient on a different occasion, all beats resemble the isolated premature beats of A in regard to P, P-R and QRST. They recur at a regular rate of 75, which is faster than the previous sinus rate. Hence, it appears that the same ectopic focus which produced the sporadic premature beats in record A has taken over the control of the entire heart in record B since its impulses occur in regular sequence and at a rate faster than the primary sinus pacemaker.

The main problem in this case is in identifying the location of the ectopic focus and determining its bearing upon the possible mechanisms of the pre-excitation syndrome. The two relevant facts in this connection are: (1) that the ventricular complex of the ectopic beats starts like the sinus beats but the final element of the sinus beats is missing; (2) that there is a retrograde premature P wave ahead of these beats with a P-R shorter than that of the sinus beats. Since the criterion of use of the normal A-V path (the slender upright terminal part of sinus QRS) is absent, it is concluded that the ordinary A-V conduction path is not being used in the ectopic beats. If this is so, then these beats cannot be nodal in origin (even though they have some of the earmarks of nodal origin); otherwise, some element of normal A-V conduction should be visible in the QRS of these beats since an impulse arising in the A-V node has to traverse it. It is thus the absence of signs of ventricular fusion in

the ectopic beats which excludes their origin in a nodal focus in this instance. The ectopic focus must therefore be localized to an area which permits: (1) rapid retrograde stimulation of the atria; and (2) stimulation of the ventricles over anomalous pathways, either completely when the ectopic focus dominates the heart, or partially when the sinus rhythm is effective. The logical conclusion is that the ectopic focus is located within an anomalous A-V bridge.

This record is significant in regard to the concept of the pre-excitation syndrome. It shows that the anomalous path not only permits impulses to bypass the ordinary conduction pathway but also can initiate impulses itself or, more properly, contains tissues which have this potential property. Ordinarily, the property of conductivity seems to be dominant in the anomalous bypass and that of impulse formation is latent or absent. If our interpretation is correct, the A-V bypass in this case showed both conductivity and spontaneous rhythmicity. The former led to ventricular pre-excitation during sinus rhythm, the latter to sporadic premature beats and a paroxysm of ectopic heart action in which retrograde conduction was as rapid as forward conduction. The demonstration of such a dual function provides further support for the assumption of an accessory A-V connection in the pre-excitation syndrome.

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Gross Hematuria in Sickle Cell Trait and Sickle Cell Hemoglobin-C Disease*

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Hematuria in sickle cell anemia has been ascribed to congestion of small vessels, thrombosis, stasis and infarction necrosis in the kidneys.[1] These changes have been linked to the abnormal sickle shape of the red cells in this disease. Pathologic evidence has been obtained in these cases of puckering of the renal surface from obliteration of engorged arteries, destruction of glomeruli from blockage of glomerular arteries, and scarring in the apices of the pyramids as a result of capillary bed blockage by sickled red cells between collecting tubules. Kimmelstiel[2] described the changes as temporary capillary stasis after shock, and spasm with ischemia and necrosis as a result of plugging of capillaries by sickled cells, resulting in vessel wall injury and, rarely, in true capillary thrombi.

The incidence of hematuria with sickle cell anemia has been reported by Henderson[3] as three in fifty-four cases and by Patterson, Wilson and Diggs[4] as two in 142 cases.

Abel and Brown[5] in 1948 reported a case of gross hematuria with a red cell count of 4,000,000, back pain and persistent filling defect in the left kidney in which eventual nephrectomy was performed. This could be considered a doubtful case of hematuria in sickle cell trait. Goodwin[6] reported four cases of hematuria in sickle cell disease, several of which could have been due to sickle cell trait. It is also possible that some of his cases may have been sickle cell hemoglobin-C disease. He also reviewed the hospital records from 1938 to 1947 and found nineteen cases of essential hematuria in Negroes, six of whom had sickle cell trait, two were negative and the rest were not tested. This author postulated minute thrombosis leading to stasis and vascular leakage as the underlying pathology. Greecy et al.[7] reported a case of sickle cell trait with hematuria, in which nephrectomy was performed. The pathologist reported a grossly normal kidney with clumping and sickling of red cells in the papillae on microscopic section. Pyelography and cultures for acid-fast organisms were negative. Ryan and Fuller[8] reported the case of a thirty-one year old Negro with sickle cell trait who had painless hematuria and in whom cystoscopy and retrograde studies were negative. Nephrectomy was performed, examination of the specimen showing small hemorrhagic areas in the mucosa of the calyces. Harrison and Harrison[9] reported on nine patients with unilateral hematuria and sickle cell disease. Most of the cases represented sickle cell trait; one had sickle cell anemia. Nephrectomy was performed in four cases and pathologic examination revealed petechiae, edematous papillae, hemorrhages, vascular dilatation and sickling in the vessels. In one case increase in size and proliferation of tubular epithelial cells was seen. The authors favored Kimmelstiel's shock theory of peripheral vascular spasm, leading to ischemic necrosis of tissue by "packing" of capillaries with sickled cells causing vessel wall injury and ultimate infarction. They also suggested an additional factor of inherent kidney defect, vascular or otherwise, in the presence of sicklemia as a cause of these changes. Lund et al.[10] reported seven cases of gross hematuria; six were unilateral and one was eventually bilateral. Two nephrectomies were performed and pathologic examination revealed sickle cells in the engorged small vessels, venules, capillaries, glomeruli and areas of hemorrhage. Their studies included acid-fast cultures, Papannicolaou and hemoglobin alkali resistance

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determinations. They found that bleeding was more common on the left side.

Recent remarkable advances in the electrophoretic study of the hemoglobinopathies have clarified many aspects of sickle cell disease.[21–23] Many of the unusual cases of sickle cell crisis without anemia, with transient anemia or with persistent splenomegaly can now be explained by the presence of sickle cell hemoglobin-C disease. In 1929 Levy[11] reported cases of sickle cell disease, one of which could easily fit this category. (Table I.) Bauer[12,13] stressed the importance of circulatory stasis from engorgement of small vessels by sickled red cells as cause of symptoms, and minimized hemolysis. Review of his case reports suggest that one of these could have been sickle cell hemoglobin-C disease. Green and Lockhard[14] reported symptomatic sickle cell disease without persistent anemia, one case with a very unusual hemoglobin pattern electrophoretically; these three cases could well have been sickle cell hemoglobin-C disease. Smith and Lockhard[15] reported the results of filter paper electrophoresis of hemoglobin in two cases of hematuria associated with sickle cell hemoglobin-C disease. Diggs and Jones[16] reported the case of a forty-eight year old Negress in whom abdominal pain, joint pains, fever, icterus, petechiae and coma developed after an alcoholic debauch, with sickling in the peripheral blood smear, targeting of the red cells and splenomegaly. It was postulated that the sickling process was precipitated by the alcoholic excess.

TABLE I

CASES IN LITERATURE WHICH MAY HAVE BEEN SICKLE CELL HEMOGLOBIN-C DISEASE

Authors	Age of Patient (yr.)	Hemoglobin	Red Blood Cell Count	Spleen	Icterus	Crises	Targeting, Sickling	Electrophoresis
Levy (1929)	8	80%	5.0	3 cm.	+	+	−−	.....
Bauer (1943)	24	78%	5.15	550 gm.	?	+	.....	.....
	26	?	?	620 gm.	..	++	.....	.....
Green and Lockhard (1951)	50	12 gm.	5.47	0	+	+	−−	Abnormal, not SS or SA
	22	11 gm.	5.64	Tip palpable	+	+	−−	.....
	24	12.3 gm	5.05	++	+	+	−−	.....
Diggs and Jones (1952)	48	11 gm.	3.12	400 gm.	+	Severe crisis after alcoholic bout	+++	.....
Smith and Lockhard (1953)	35	.....	3.8	+	−	Hematuria	.....	SC − FH 3.8%
	16	.....	....	.....	..	Hematuria, knee pains	.....	SC − FH 6%

A summary of these possible instances of sickle cell hemoglobin-C disease, as reported in the literature, is given in Table I. Five additional cases of sickle cell trait and three of sickle cell hemoglobin-C disease manifesting gross hematuria are described in this communication. Filter paper electrophoresis and fetal hemoglobin studies were performed. (Fig. 1.) Urologic studies and results of nephrectomy in several of these cases are being reported separately in more detail.[18]

CASE REPORTS

CASE I. C. A., a thirty-eight year old man, was admitted to the Hines V. A. Hospital in June, 1953. He stated that he had had a bout of gross hematuria in September, 1950, treated at

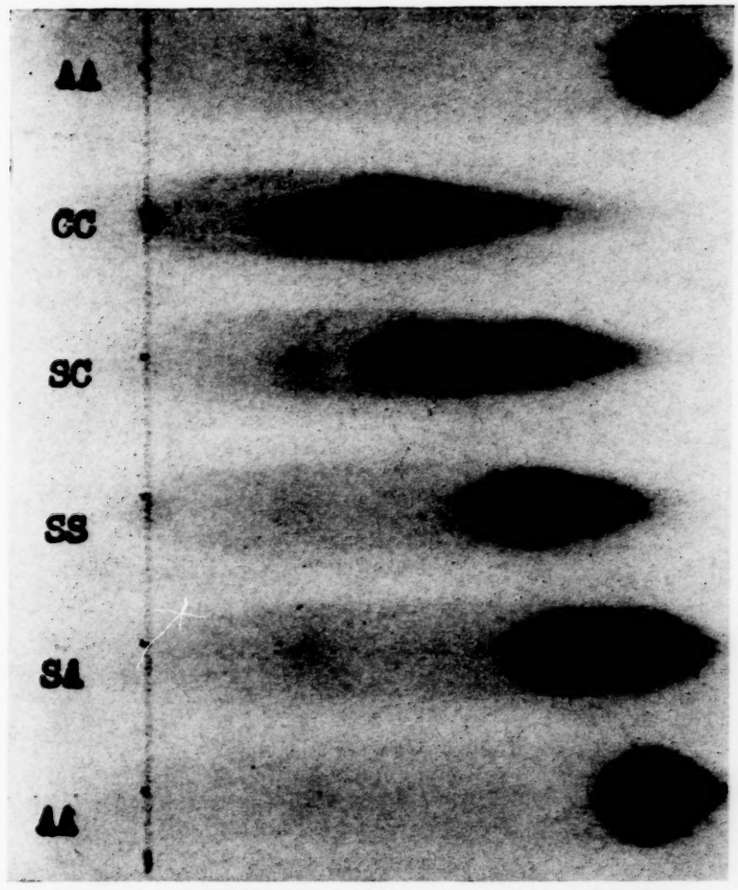

FIG. 1. Photograph of filter paper strip showing types SA and SC hemoglobins as seen in our patients. Types SS, AA, and CC hemoglobins are shown for comparative purposes.

the V. A. Hospital in Memphis, Tennessee. There were no further episodes of hematuria until June, 1953, when he noted dark urine for one week followed by grossly red urine one week preceding admission to the hospital. Physical examination was not remarkable. Hepatosplenomegaly was not present. The sickle cell test was positive; fetal hemoglobin was normal; filter paper electrophoresis revealed type AS hemoglobin. Evidence of hemolysis was lacking; blood chemical studies and genitourinary examinations were normal. Since his discharge from the hospital in June, 1953, the patient has had two episodes of hematuria to date. His last red blood cell count was 4,700,000, with 14 gm. of hemoglobin. Subsequent urologic examinations have been negative.

CASE II. J. G. B., a thirty year old Negro man, was first hospitalized at the Hines V. A. Hospital in 1949, on the Genitourinary Service. He gave a history of recurring episodes of bloody urine since 1944. Because of abnormal intravenous and retrograde pyelograms, exploration of the left kidney was performed but no abnormalities were found.

In June, 1951, he was seen on follow-up examination and a sickle cell preparation was found to be positive. He has had frequent episodes of hematuria since his original admission in 1949. In all instances the hematuria has been painless and has cleared spontaneously. Physical examination during a recent hospitalization revealed a well developed, well nourished, Negro man with no abnormal physical findings. Laboratory studies showed a red blood count of 3,800,000, with 12.5 gm. of hemoglobin. The urine has varied between grossly bloody and negative. Chest x-rays have been essentially normal. Filter paper electrophoresis revealed type AS hemoglobin. Alkali denaturation technic for fetal hemoglobin was normal. Subsequent intravenous pyelograms have been normal and

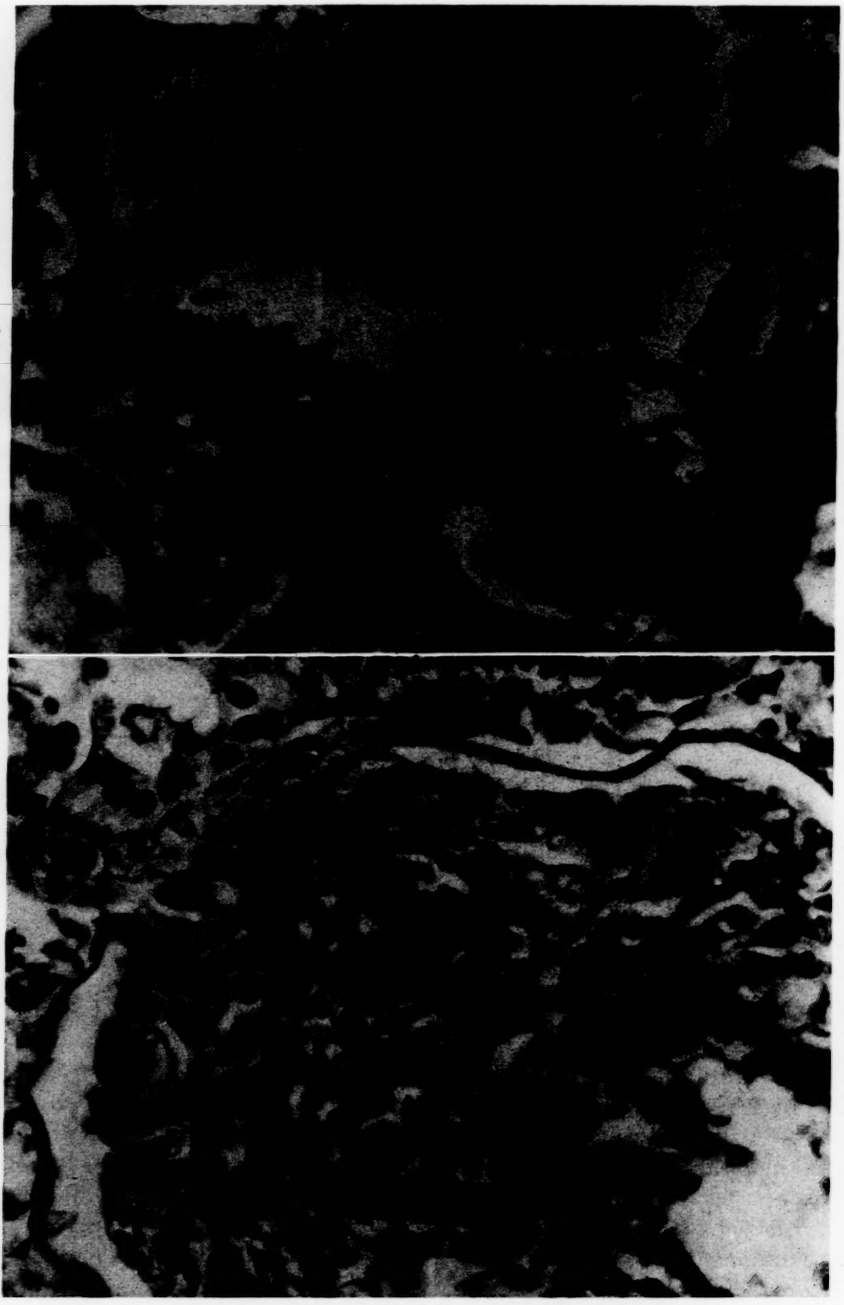

FIG. 2. A and B. Photomicrographs of sickle cells. A, in renal tubules; B, in glomerulus of kidney in one case of gross hematuria.

urine culture for acid-fast bacillus have been negative.

CASE III. J. L., a twenty-four year old Negro man, first noted gross hematuria in November, 1948, when he was serving in the Army as a laboratory technician. An intravenous pyelogram taken at this time was negative. In March, 1949, complete genitourinary studies, including urine cultures, intravenous pyelograms and retrograde pyelograms, were carried out and a defect was found at the left ureteral pelvic junction suggestive of extrinsic pressure. Cystoscopic findings revealed fresh blood coming from the right ureter. The patient continued to have gross and constant hematuria. In August, 1949, right nephrectomy was performed. The patient

thereafter noted no hematuria and was well until August, 1952, when he was hospitalized at the Hines V. A. Hospital for gross painless hematuria. Throughout his hospital stay he showed alternating gross and microscopic hematuria; blood transfusion was necessary because of a low red cell count at one time during his hospital stay. Physical examination revealed a well developed, well nourished, Negro man who did not appear acutely ill. The heart and lungs were normal. There was no enlargement of the liver or spleen. Laboratory findings revealed a normal hemogram. The platelet count was 182,000. The sickling test was positive with metabisulfite. Filter paper electrophoresis revealed type AS hemoglobin. Alkali denaturation showed 0.8 per cent fetal hemoglobin. Frequent urinalysis during this hospitalization revealed grossly bloody urine. Retrograde and intravenous pyelograms were negative. A review of the sections obtained at the time of nephrectomy showed normal kidney parenchyma. Sickling of the red blood cells was noted in the vascular lumina. There were also sickled red blood cells in the ureteral wall associated with a submucosal hemorrhage.

During this patient's last period of hospitalization he became asymptomatic. The hematuria ceased and he was discharged in December, 1953. On follow-up examination he reports no further hematuria.

Case IV. J. P., a seventeen year old Negro man, was admitted to the Cook County Hospital on May 27, 1953, with a history of painless gross hematuria of two weeks' duration. Cystoscopic study revealed bleeding from the left kidney but the pelvis was found to be normal after pyelogram studies. He was then asymptomatic for a period of three weeks but gross, painless hematuria recurred and he was readmitted to the hospital. There was no history of chills, fever or weight loss. There was a history of tuberculosis in the family. Physical examination revealed a well developed, well nourished, Negro man in no acute distress, and was essentially negative except for small bilateral axillary nodes. Chest x-ray was negative. The Mantoux test was positive in dilutions of 1:1,000 in forty-eight hours. Urine examination revealed gross hematuria. A sickle cell preparation was positive in twenty-four hours by the sealed technic. Hemogram and fragility studies were normal. Bone marrow revealed accelerated erythropoiesis. Paper electrophoresis demonstrated type AS hemoglobin. Fetal hemoglobin by alkali denaturation was 1.2 per cent. Genitourinary study was negative except for the observation of bleeding from the left ureter.

This patient was seen on follow-up examination in September, 1953, and at that time gross hematuria continued. There were no other complaints. No enlargement of the liver or spleen was noted. The eyegrounds were negative. Cultures of the urine for acid-fast organisms were negative.

Case V. C. H. G., a twenty-seven year old Negro man, was first admitted to the Hines V. A. Hospital in 1948 because of a detached retina which occurred while he was in service. At this time a sickle preparation was positive. He was readmitted to the Genitourinary Service in July, 1953, with gross hematuria. Evaluation of the genitourinary tract at that time was considered normal. He was again admitted to the Genitourinary Service in November, 1953, because of a second episode of painless, gross hematuria. Examination revealed a well developed, well nourished Negro man who was in no acute distress. The spleen was palpable 1 cm. below the left costal margin on deep inspiration. The remainder of the examination was essentially normal. Retinitis proliferans of the right eye was found by consultants from the Eye Department; this was thought to be secondary to an old retinal detachment and hemorrhage. Laboratory findings were as follows: Urine cultures for acid-fast bacilli were negative. Retrograde pyelogram revealed filling abnormalities in the right middle calyx suggestive of clots in the pelvis of the kidney. Cystoscopy revealed blood coming from the right ureteral orifice. X-ray of the chest was negative. X-rays of the ribs revealed a general increase in width with slight increase in the trabecular patterns suggestive of the bone changes in sickle cell anemia. The hemogram was normal as was the fragility test. Reticulocyte count was 1.5 per cent. Sickling was positive by the sodium metabisulfite technic. Fetal hemoglobin determination by alkali denaturation technic revealed 1.3 per cent. Filter paper electrophoresis revealed type SC hemoglobin.

The patient was placed at bedrest, had an uneventful recovery and was discharged from the hospital. Subsequent follow-up examinations have not revealed any further evidence of hematuria.

Case VI. D. P., a forty-one year old, Negro man, was admitted to the Hines V. A. Hospital

on June 28, 1952, with a two-month history of painless and periodic gross hematuria, accompanied by general weakness over the same period of time. He gave no history of prior episodes of hematuria, melena or hematemesis. Physical examination was not remarkable, revealing a moderately well nourished, well developed, Negro man. He was readmitted to the hospital on December 30, 1953, because of gross hematuria, with clots, of three days' duration. Cystoscopy at this time revealed a bloody efflux from the left ureteral orifice. Intravenous pyelograms and renal function tests were normal. Physical examination at this time was essentially negative except for enlargement of the liver which was palpated 2 cm. below the right costal margin. The spleen was not enlarged to palpation. Laboratory findings revealed a normal hemogram. Many target cells were noted in the peripheral smear. Sickling was positive by sodium metabisulfite technic. Fetal hemoglobin was 4 per cent. Filter paper electrophoresis revealed type SC hemoglobin. Phenolsulfonephthalein excretion was 45 per cent in fifteen minutes.

Hematuria persisted until January, 1954, lasting a total period of two weeks, and then stopped abruptly and did not recur. Subsequent follow-up examinations have shown no evidence of hematuria.

Case vii. J. G., a twenty-five year old Negro man, was admitted to the Hines V. A. Hospital on November 19, 1951, because of painless gross hematuria, with clots, of three days' duration. He complained of nocturia two times a night, but denied chills, fever, weight loss or night sweats. There was no history of venereal disease, urethral instrumentation or tuberculosis. Physical examination revealed a moderately well nourished Negro man. He complained of weakness and light-headedness. The spleen was palpated 6 cm. below the left costal margin and was non-tender, smooth and firm. The liver was not palpable. The remainder of the physical examination was not remarkable.

The hemogram showed the red blood count to be 4,000,000 with 13 gm. of hemoglobin. Platelet count and prothrombin time, serum non-protein nitrogen and blood proteins were normal. Red cell fragility studies were negative, as were the liver function tests. No sickling was noted on direct smear but increased numbers of target cells were found. The patient was cystoscoped and a bloody efflux was noted coming from the left ureteral orifice. Genitourinary studies revealed normal retrograde pyelograms but intravenous pyelograms revealed a filling defect in the left upper calyx suggestive of clots in the pelvis. On December 12, 1951, because of persistent, gross bleeding from the left kidney of four weeks' duration and with evidence of poor filling in the upper left kidney and calyx, left nephrectomy was performed. Grossly the kidney had a smooth surface and was pale yellow in areas. There was considerable perirenal fat. Mucosal erosion of the papillae and petechial hemorrhage were present. Microscopic examination revealed the renal architecture to be preserved. Glomeruli and tubules appeared to be essentially normal. One section of the ureter revealed an erosion of the mucosa, underneath which there was severe hyperemia at the site of bleeding. Sickling of red blood cells was observed in the vessel lumina and in the area of the submucosal hemorrhage. Repeat sickling procedures with sodium metabisulfite revealed a positive sickling test. Filter paper electrophoretic studies demonstrated type SC hemoglobin. Alkali denaturation studies revealed a fetal hemoglobin of 1.3 per cent. The patient was seen once subsequent to his operation at which time hematuria had recurred. This is the only episode of hematuria that he has had.

Case viii. D. W., a twenty-nine year old Negro man, was admitted to the Hines V. A. Hospital in December, 1953, with a history of gross hematuria of several days' duration. The hemogram at this time was normal, and a smear for sickling was reported as negative. No further hematologic investigation was made at that time. Intravenous and retrograde pyelograms were normal and cultures of the urine for acid-fast bacilli were negative.

The patient was readmitted in June, 1954, for follow-up examination and the metabisulfite technic for sickling was found to give a positive result. Filter paper electrophoresis revealed type AS hemoglobin. The hemogram was normal as were reticulocyte counts, platelet counts and urinalyses. He has had no further episodes of hematuria.

Summaries of the hematologic and urologic findings in these cases may be found in Tables ii and iii.

COMMENTS

A review of hospital records (U. S. Veterans Administration Hospital, Hines, Illinois) over a period of four years (1950 through 1953)

revealed seventy-six cases of idiopathic gross hematuria, twenty-two of which were in Negro patients. In the latter group, six were studied by the authors and found to have positive sickling tests. The remaining sixteen were recalled for evaluation and each of these patients had completed genitourinary studies, including intravenous and retrograde pyelograms and urine cultures for acid-fast bacilli. Two patients of this recall group had had nephrectomies because of persistent hematuria. A review of the kidney sections revealed the presence of sickled red blood cells in the lumina of the vessels of one of these cases. Peripheral smear studies for sickle cells prior to his surgery had been reported negative. He has not been available for metabisulfite sickling tests or filter paper electrophoresis studies at this time and for this reason was not included in the present group of patients. Of the remaining fourteen patients, five have been located and re-examined by us and one (Case VIII) has been found to have a sickle cell trait. The remaining four did not exhibit sickling with sodium metabisulfite and electrophoretic patterns revealed homozygous hemoglobin A. Our fourth case was found at the Cook County Hospital.

The generally accepted incidence of sickle cell trait in the Negro population is approximately 8 per cent.[19] We believe that the incidence of unexplained hematuria associated with sickle cell trait, which we have noted in six of fourteen patients available for study, is significant and cannot be ascribed to mere coincidence. It would appear that the incidence of hematuria is more frequent with sickle cell trait than in sickle cell anemia. The occurrence of sickle cell anemia being about 0.3 per cent of the Negro population,[19] the age distribution and life expectancy with this disease may account for the difference.

TABLE II
SUMMARY OF HEMATOLOGIC FINDINGS

Patient	Hemoglobin Type	Fetal Hemoglobin (%)	Sickling		Target Cells	Red Blood Cell Count (million)	Hemoglobin (gm.)	Reticulocytes %	Van den Bergh Test (mg. %)	Splenomegaly	Fragilities	
			Direct Smear	Bisulfite							Initial	Complete
C. A.	AS	1.0	0	+	0	5.0	14	0.1	0.3/0.6	0	36 47	26 30
J. G. B.	AS	1.5	0	+	0	4.7 to 3.8	14	0.2	0.4/0.8	0	40	30
J. L.	AS	0.8	0	+	+	4.5	14	...	0.4/0.9	0	36	22
J. P.	AS	1.2	0	+	0	4.3	.....	...	.....	0	36	24
C. H. G.	SC	1.3	0	+	+++	4.5	13	1.5	0.7/1.3	+	36	24
D. P.	SC	4.0	0	+	+++	2.7 to 4.4	8 to 13	...	0.15/0.5	0	36	24
J. G.	SC	1.3	0	+	++	4.5	15	...	0.15/0.5	++	44	24
D. W.	AS	2.1	0	+	0	4.0	12	0.1	.....	0	..	..

At the beginning of our study, before electrophoresis was available, our cases were considered clinically to be examples of hematuria with sickle cell trait. It was soon noted that several cases were sickle cell hemoglobin-C in type. Clinically, since sickle cell hemoglobin-C disease is associated with symptoms and findings more closely resembling sickle cell anemia than sickle cell trait, we naturally anticipated that most of our cases would fall in the sickle cell hemoglobin-C group. Certainly, hematuria would be expected in the more active form of sickle disease. We believe, therefore, that the occurrence of hematuria in patients with sickle cell trait is perhaps more significant than has been previously realized.

In the three cases of sickle cell hemoglobin-C

disease with hematuria, all had targeting of the red cells, two had splenomegaly and one had evidence of hemolysis and bone changes. None had a history of crisis or exhibited the body build and other features of sickle cell anemia.

All of our cases with sickle cell trait displayed typical electrophoretic patterns, insignificant fetal hemoglobin levels and had no anemia or evidence of hemolysis. Every effort was made by repeated studies to exclude possible genitourinary disease, systemic disease or coagulation defects which could have been responsible for hematuria in these patients. Consequently, we feel justified in linking the hematuria with the presence of sickle cell trait in these patients.

Evaluation of the clinical course of our pa-

TABLE III
SUMMARY OF GENITOURINARY FINDINGS

Patient	Hematuria	Pyelography		Cystoscopy	Cultures	Surgery	Course and Duration
		Intravenous	Retrograde				
C. A.	Gross	Normal	Normal		Negative	None	Onset 1950; has had four episodes of gross hematuria in past four yr.
J. G. B.	Gross	Probable clots in left renal pelvis, 1949; subsequent I.V.P. normal, 1951	Normal	Bleeding from left ureter	Negative	Exploratory laparotomy; normal-appearing kidney and ureter	Onset 1944; has had frequent episodes of gross, painless hematuria clearing spontaneously
J. L.	Gross	Normal, 1948; defect at left ureteropelvic junction, 1949; normal, 1952	Normal, 1948 Normal, 1952	Bleeding from right ureter	Negative	Right nephrectomy, 1949	Onset 1948; frequent episodes of painless bleeding; persistent bleeding until nephrectomy in 1949; sporadic since
J. P.	Gross	Normal	Normal	Bleeding from left kidney	Negative	None	Onset 1953; two episodes of painless gross hematuria
C. H. G.	Gross	Normal	Abnormal filling defects in renal pelvis, thought to be clots on right side	Bleeding from right ureter	Negative	None	Onset, 1948; two episodes of gross painless hematuria in July and Nov., 1953
D. P.	Gross	Normal	Normal	Bleeding from left ureter	Negative	None	Onset 1952; two episodes of gross painless hematuria
J. G.	Gross	Questionable filling defects, suggestive of clots on left	Normal	Bleeding from left ureter	Negative	Left nephrectomy, 1951	Onset 1951; persistent gross hematuria until left nephrectomy; recurrence of hematuria on one occasion since surgery ceased spontaneously
D W.	Gross	Normal	Normal		Negative	None	Onset 1953; one episode of gross, painless hematuria

tients with hematuria revealed a significant absence of pain in association with the episodes of hematuria. All but one patient has had repeated episodes of gross hematuria which subsided spontaneously, including the two patients in whom nephrectomy was performed. When cystoscopy reports were available it was noted that the bleeding was more frequently on the left side, as has been observed by Lund.[10] In the two patients undergoing nephrectomy surgery was performed only after prolonged gross bleeding and it is noteworthy that hematuria recurred in both cases. All other patients were treated primarily with bedrest.

The pathologic findings in our two nephrectomized patients revealed similar changes; namely, submucosal hemorrhage, sickling of the red cells in the vessel lumina with engorgement of these vessels as well as of the capillaries of the glomeruli, and preservation of the normal renal parenchyma. One had ureteral erosion with underlying hyperemia. No thrombosis was seen in any of the microscopic sections reviewed.

The most challenging feature of the association of sickle cell trait with hematuria has been the underlying etiologic mechanism of this syndrome. Since sickle cell trait is not generally accompanied by the usual stigmata of sickle cell anemia, such as crisis, hemolytic anemia, bone changes or unusual body build, we could postulate local tissue insult as the trigger mechanism for vascular injury and subsequent bleeding. Vascular defects of the retina have been described in sickle cell trait by Henry and Chapman.[20]

The kidney is particularly susceptible to the effects of shock, infection and ischemia. Therefore, it is not unreasonable to assume that local changes in the kidney due to such factors might be responsible for the anoxia precipitating the sickling phenomena, with subsequent vascular changes and bleeding. The singular susceptibility of kidney and retina to stress might well account for the unexpected pathologic changes in an otherwise clinically benign condition.

SUMMARY

1. The occurrence of gross hematuria in five cases of sickle cell trait and three cases of sickle cell hemoglobin-C disease is reported. The investigation of these patients included electrophoretic and fetal hemoglobin studies.

2. Some of the previous cases of sickle cell trait reported with symptoms of crisis and/or hematuria might well have been sickle cell hemoglobin-C disease. The literature is reviewed in this connection.

3. The finding of hematuria with sickle cell trait in a significant number of patients is believed to indicate a significant relationship.

4. The singular susceptibility of the kidney to stress associated with sickle cell trait is offered as a possible etiologic mechanism for the vascular defects causing hematuria.

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Adrenal Hormone Therapy in Viral Hepatitis*

IV. The Effect of Gamma Globulin and Oral Cortisone in the Acute Disease

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In preceding studies[1,2] it was shown that the use of ACTH and cortisone resulted in definite improvement in cases of acute viral hepatitis treated with these agents. There was a prompt fall in serum bilirubin and increase in appetite with both drugs. Cortisone in particular brought about a primary recovery period approximately two weeks shorter than did a group of similar controls, and histopathologic studies revealed more rapid progress toward healing. The two principal disadvantages of this form of treatment were a relapse rate of 20 per cent in the treated cases as compared to none in a group of similar controls, and the length of treatment (twenty-eight days) which was prolonged and painful since the intramuscular route was used. Although this therapy had its advantages, it left the patient more vulnerable to relapses, due perhaps to disturbances in virus-host relationships important to immunity.

It was believed that immunity might be bolstered by the concurrent use of gamma globulin, and that treatment might be rendered easier by the oral use of cortisone over a shorter period of time. Values for gamma globulin measured as units of zinc sulfate turbidity had shown a definite decrease with both ACTH and cortisone in previous studies[1,2] which lent further credence to the possibility that additional amounts of this material, given during the course of treatment, might be beneficial. Gamma globulin has been well established as an effective agent in the prevention of infectious hepatitis but its use for the treatment of both acute[3] and chronic[4] viral hepatitis is of questionable value when combined with standard rest and dietary regimens. Its specific application to the problem of enhancing immune mechanisms in patients receiving ACTH or cortisone has not been previously attempted.

MATERIALS AND METHODS

Plan of Study. Three groups of patients were studied, the first two groups consisting of ten patients each, and the last of two patients only, due to time limitations and scarcity of suitable material. These patients, designated as Groups I, II and III, were given oral cortisone and intramuscular gamma globulin according to the following schedule:

Group I received 10 cc. of gamma globulin on the day treatment with cortisone was started, and the same amount one week later. Cortisone was given in amounts of 300 mg. the first day, 200 mg. the second day, 100 mg. the third and fourth day, 80 mg. the fifth, 40 mg. the sixth and 20 mg. the seventh day.

Group II received 30 cc. of gamma globulin at the start of therapy and an additional injection of 20 cc. on the fortieth day of disease. Since all patients were started on this treatment between the tenth and twentieth day of disease, there was little individual variation of the time interval between the first and second injection. Cortisone was administered over a two-week

* From the Hepatitis Center, 98th General Hospital, U. S. Army, Europe. The present study represents a continuation of investigations of viral hepatitis at the Hepatitis Center in Europe which were begun in 1947 under the sponsorship of the Commission on Virus and Rickettseal Diseases, Armed Forces Epidemiological Board, Office of the Surgeon General, U. S. Army, Washington, D. C.

period, 300 mg. the first day, 200 mg. the second and third days, and 100 mg., 75 mg. and 50 mg. for successive periods of three days each, followed by a final two days of 25 mg. each.

Group III was given gamma globulin injections according to the same schedule as Group II.

probable that the majority of our patients had infectious hepatitis.

Controls. Statistics from control groups collected in previous studies were used[1,2] as they were believed to be recent and sufficiently reliable to be valid.

TABLE I

PRETREATMENT VALUES FOR PREVIOUSLY STUDIED AND PRESENT GROUPS*

Group	No. of Patients	Age	Day of Disease†	Serum Bilirubin	
				Total (mg. %)	One Minute (mg. %)
Previously studied					
ACTH	10	22.9	14.6	10.9	5.8
Alternate control*	10	22.5	13.9	9.6	4.9
Prior control	100	24.1	14.0	9.1	4.8
Gamma globulin + cortisone					
Group I	10	23.6	12.3	12.0	6.1
Group II	10	22.3	15.1	11.3	5.1
Group III	2	22.0	16.0	9.0	4.9

* The alternate control group represent patients given placebo injections in alternation with patients receiving ACTH in a previously reported study.[1]

† Day of disease indicates day when hormonal therapy or control injections were started and day of admission laboratory studies for the prior control group.

Cortisone was administered over a three-week period on a somewhat higher average dosage schedule as follows: 300 mg. the first three days, 200 mg. the next four days, 150 mg. for five days, 100 mg. for five days, and 75 mg. and 50 mg. for two days each.

Criteria for All Groups. (1) Uncomplicated viral hepatitis as based on typical history, physical and laboratory findings; (2) demonstrably rising total serum bilirubin over 5.00 mg. per cent prior to the start of therapy; (3) onset of disease not more than twenty or less than ten days previous to therapy; (4) absence of contraindications to cortisone therapy; and (5) voluntary consent for liver biopsy following treatment. The period of ten to twenty days after onset of disease was chosen for institution of hormone therapy rather than an earlier period because our former experience[1,2] suggested that such delay might reduce the relapse rate and in addition it eliminated those patients in whom rapid recovery occurred without hormone treatment. It was not possible to determine whether the type of viral hepatitis encountered was due to virus A or virus B, although it is

Biopsies of the Liver. These were performed between the thirty-fifth and fortieth days of illness. None were done prior to therapy since it was thought that previous material was ample for comparison and that study following therapy at this time interval would serve to establish the diagnosis as well as effects of therapy and would be comparable in timing to previous studies in which controls were used. Adequate specimens were obtained in all cases and no complications were encountered.

Observations. Clinical observations were recorded on a standard form and laboratory procedures including complete liver function studies weekly or oftener followed the pattern of preceding studies,[1,2] with the exception that electrocardiograms were not performed routinely.

A high protein, 4,200 calorie low salt diet and absolute bedrest were prescribed as before.[1,2]

PRETREATMENT STUDIES

The status of patients immediately prior to institution of oral cortisone-gamma globulin therapy given in Table I is compared with similar pretreatment data for previous patients in a

similar period of illness (second ten days after onset) who had been given ACTH or served as controls.[1] The age of the patients and duration of disease at the start of therapy or the control period were quite similar for all groups. The total serum bilirubin of Groups I and II of the present study were 2 to 3 mg. per cent higher, however, than the control groups. This has some bearing on the results to be presented since the intensity of jaundice is one expression of the severity of disease and of the expected duration of the hepatitis. Not included in Table I are previous patients started on ACTH or cortisone therapy in the first ten days of illness.

TABLE II*

COMPARISON OF RESULTS IN GAMMA GLOBULIN–CORTISONE STUDY GROUPS WITH PREVIOUSLY TREATED AND CONTROL GROUPS

Groups	No. of Cases	Duration of Hormone Therapy (days)	Day of Disease when Test Returned to Normal		Relapses in Group
			Total Serum Bilirubin	BSP Retention (10% or less)	
Previously treated					
ACTH	10	28	44.1	55.0	0
Alternate control	10	0	55.1	57.3	0
Prior control	100	0	55.1	59.4	0
Gamma globulin + cortisone					
Group I	10	7	49.9	60.3	2
Group II	10	14	46.9	53.1	2
Group III	2	21	32.5	46.5	0

* This table includes only patients started on therapy or control period ten to twenty days after onset of symptoms. Those previously placed on the program in the first ten days of illness using ACTH[1] or cortisone[2] are omitted.

RESULTS OF THERAPY

Patients treated with oral cortisone had a rapid and often dramatic fall in serum bilirubin within the first few days of therapy and this was usually accompanied by an increase in appetite and well-being. The effects were similar to those previously observed with parenteral cortisone or ACTH.

The duration of illness measured by the day of disease on which the total serum bilirubin (TSB) reached normal (1.0 mg./100 cc.) and the bromsulfalein retention (BSP) reached 10 per cent (forty-five minute sample) or less is compared in Table II with results of our earlier studies. Patients given oral cortisone for one or two weeks did not recover much more rapidly in terms of these criteria than control patients despite their good initial response. That this may be related to the duration of hormone therapy is suggested by the shorter duration of illness in the two subjects receiving three weeks of therapy in whom results were comparable to those of parenteral cortisone given for four weeks in which the TSB and BSP became normal on the thirty-first and forty-second day of illness, respectively.[2]

One other factor bears on these results. The experience at this Center with more than 5,000 cases of viral hepatitis has emphasized that although the duration of disease is unpredictable in an individual patient, good correlation exists in large groups of patients between the height of bilirubinemia and the total duration of jaundice.[5] The serum bilirubin at the start of therapy in patients in the present study was sufficiently higher than in control patients to merit analysis from this standpoint. This has been done in Figure 1 in which data are included on ten patients started on ACTH at a comparable time after onset of illness. The results indicate that the total period of bilirubinemia was proportionally shortened by both oral cortisone and parenteral ACTH therapy as compared to control groups. We hold no brief that this effect necessarily indicates recovery from hepatitis but wish to emphasize that adrenal hormone therapy significantly influences the disappear-

ance of jaundice. Just how this occurs is unknown. It might operate through extrahepatic mechanisms such as increasing renal excretion or inhibiting a hypothetic hemolytic factor. A direct hepatic action is far more likely, however, The more rapid histologic healing in liver

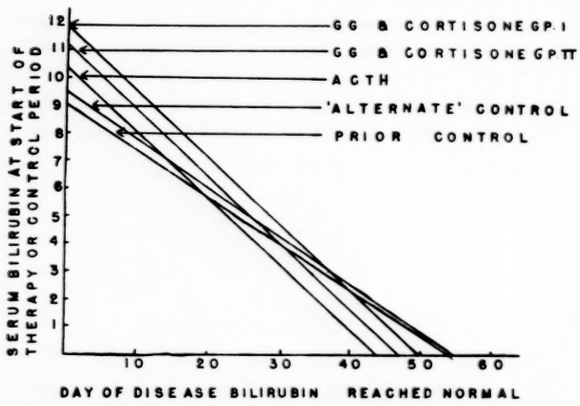

FIG. 1. Rate of return of total serum bilirubin to normal. In this chart only patients are included who were started on hormonal therapy or control period ten to twenty days after onset of illness.

biopsies from patients given cortisone over a four-week period[2] supports this.

EFFECT OF CORTISONE ON LIVER FUNCTION

Serum Bilirubin. As already indicated, all twenty-two patients treated with cortisone showed typical prompt initial drops in total serum bilirubin values similar to those noted previously for this type of therapy.[1,2] There was considerable irregularity in the "leveling out" phase after the initial drop, especially in Group II. In general, results were comparable to previous studies.

Bromsulfalein Excretion. Values for this test were quite similar to control groups except in the two patients of Group III who showed a better response after receiving cortisone therapy for twenty-one days.

Other Liver Function Tests. Since such tests as thymol turbidity, cephalin cholesterol flocculation, alkaline phosphatase and cholesterol ester per cent showed little variation from controls previously,[1] and since individual variations appeared to be lacking during this study, the values were not summarized. Gamma globulin, as shown by zinc sulfate turbidity values, showed wide variations which precluded accurate graphing. There was no instance, however, of elevation of values following injection of human serum globulin and, in general, values showed mild to moderate lowering during the course of treatment, following previously noted trends.

RELAPSES

The term "relapse" has been defined for the purposes of these studies as the recurrence or recrudescence of clinical signs and symptoms of hepatitis confirmed by definite changes in liver function after an initial period of improvement.[1] By these criteria gamma globulin did not prevent relapses. Two occurred in Group I (ten patients), two in Group II (ten patients) and none in Group III (two patients). This relapse rate of approximately 20 per cent is similar to that of patients treated with ACTH or cortisone over a longer period but not in conjunction with gamma globulin.[1,2,6] All four relapses of the present series occurred at the time cortisone was discontinued. There were no "late" relapses. In four patients the relapse was characterized by a second peak of bilirubinemia higher than the first and by anorexia, malaise and abdominal pain. Relapses of this type as well as some encountered either during therapy or some time after discontinuance of therapy have been previously reported.[1,2,6]

Two other cases of the present group did not fulfil our criteria for relapse but their courses were not smooth and the response to therapy was not ideal. In the first an asymptomatic increase in bilirubinemia occurred at the cessation of therapy. In the second, erratic increases in bilirubinemia were seen during cortisone therapy. These responses, while not true relapses, probably delayed convalescence.

None of the relapses were retreated with ACTH or cortisone so that the course of disease could be observed under such circumstances. It is known that they do, in fact, respond satisfactorily to reinstitution of therapy.[6–8]

SIDE EFFECTS

Response to cortisone in the three groups is shown in Table III. The high per cent of glycosuria is consistent with previously observed disturbances in carbohydrate metabolism, although hyperglycemia was not noted. Eosinophil response was initially good in a high per cent, although later rise was noted in most patients under treatment.

CHANGES IN LIVER HISTOPATHOLOGY

The biopsy material was processed in the manner outlined in other communications.[1,2] All slides were examined as unknowns. The group of control cases was the same as before.[1,2] The biopsies were obtained from thirty-one to fifty-six days after the onset of the disease: all twenty-two showed evidence of a diffuse hepatitis consistent with the diagnosis of viral hepatitis.

TABLE III
SIDE EFFECTS OF CORTISONE AND GAMMA GLOBULIN THERAPY

	Incidence (%)		
	Group I (10 Cases)	Group II (10 Cases)	Group III (2 Cases)
Glycosuria	70	100	100
Euphoria	10	30	50
Rounding of face	30	100	100
Eosinopenia	90	90	100
Acne	20	50	100
Hyperglycemia	0	0	0
Hypertension (over 140/90)	10	0	0
Edema	10	0	0

There was good correlation between the clinical course and the histologic findings. Corresponding to the interval between the onset of clinical relapse and the time the liver biopsy was obtained, three cases showed evidence of hepatitis in an acute, active phase. In the remaining nineteen cases the clinical course was favorable in twelve, with steady progression toward cure and without any evidence of chemical or clinical recurrences or recrudescences. The section of liver in eleven of these twelve cases showed evidence of a subsiding hepatitis with minimal to slight activity, findings fully within the range expected at the time of biopsy. Only one showed evidence of a more marked, moderately severe activity. Six of seven other cases in which there had been some slight secondary rises of serum bilirubin or more prolonged abnormality of liver function tests showed greater severity of histologic lesions which, however, were still considered to be in the upper range expected for this stage of the disease process.

Excluding the patients who relapsed, there was no significant difference between the liver biopsies of patients given cortisone and those untreated. Similarly, the variation between biopsies in Groups I, II and III was not greater than the individual variations within the group itself. Fatty metamorphosis of liver cells was never conspicuous in biopsies from patients who had been treated and did not exceed that of control patients. These results are in contrast with those observed when parenteral cortisone was administered for four weeks.[2] Here the healing process was uniformly more rapid than in controls but was accompanied by fatty metamorphosis. Such differences are probably the consequence of more prolonged hormonal therapy.

DISCUSSION

Frequent relapses have been a major disadvantage of treatment of viral hepatitis with adrenal hormones and may have resulted from hormone-induced disturbances in the development of immunity.[1,2] One of the primary purposes of the present study was to determine if the administration of gamma globulin could enhance the level of immunity sufficiently to prevent relapse. No such protection occurred, as evidenced by a 20 per cent relapse rate similar to that in ACTH- or cortisone-treated patients not receiving gamma globulin.

Infectious hepatitis may be effectively prevented by gamma globulin injections given after proved exposure to this disease but serum hepatitis is not prevented in this way even when the gamma globulin is prepared from the blood of volunteers convalescent from known serum hepatitis.[9] These observations may bear on the present study. Gamma globulin would not be expected to enhance immunity to serum hepatitis since it does not prevent the disease, yet it might be effective in infectious hepatitis. Possibly the relapses encountered in this investigation occurred only in patients with serum hepatitis. This seems unlikely but since it has not been possible to differentiate infectious from serum hepatitis on clinical or epidemiologic grounds in this study[10] this question cannot be answered directly.

Oral cortisone appears to be fully as effective as intramuscular cortisone in alleviating symptoms, stimulating appetite and enhancing the disappearance of jaundice. The best results have been obtained when cortisone is given for three or preferably four weeks. There is evidence from our previous study that even histologic healing of the liver may be accelerated under such management.[2]

Despite these results we strongly believe that neither cortisone nor ACTH has any place in the routine management of viral hepatitis. The beneficial effects are more than offset by the frequency and severity of relapses. Furthermore when the average patient with hepatitis is put to bed and given a nutritious diet, recovery is usually uneventful and without sequelae. Adrenal hormone therapy does not shorten the course of total illness sufficiently to merit the risk of relapse, the expense of treatment and the possibility of unpleasant side effects.

Two situations deserve consideration for cortisone therapy. The first of these is fulminant hepatitis with coma. In over 5,500 cases of viral hepatitis studied at the Hepatitis Center, approximately sixteen patients developed definite coma.[5,6] Of these, five received ACTH, two received cortisone and the rest were treated with supportive therapy only. Of the sixteen patients only one recovered and she received massive cortisone therapy (1 gm. daily).[11,12] One other patient with very severe and rapidly progressive hepatitis in pre-coma also recovered while receiving large doses of cortisone.[6] Since leaving the Hepatitis Center, the authors have treated two other definitely comatose hepatitis patients with massive cortisone therapy, one of whom recovered.* Ducci and Katz have reported the recovery of five comatose hepatitis patients given similar therapy.[13,14] Coma due to viral hepatitis therefore represents a clear-cut indication for a trial of cortisone therapy in very large doses.

The other situation in which cortisone merits trial is the severe, rapidly progressive case of marked jaundice (over 15.0 mg. per cent) in which anorexia and vomiting have not responded to intensive conservative therapy with intravenous glucose. Our experience with ACTH in this situation has been unfavorable, attended as it was by frequent relapses of major and minor severity and occasional severe side effects[6] but recent trials with cortisone under these conditions have been more promising. The attendant danger of relapse is ever present, however, and one must reinstitute therapy promptly if this occurs. Cortisone should probably be continued for a period of not less than three to four weeks, preferably until the serum bilirubin reaches normal, and then discontinued gradually. The advantages and disadvantages of such management should be weighed carefully against the seriousness of the patient's condition.

* The fatal case is included in the references,[6] the other is unreported.

SUMMARY

1. Twenty-two patients with acute viral hepatitis were given oral cortisone in conjunction with the administration of large amounts of gamma globulin intramuscularly at the beginning and end of hormonal therapy.

2. Gamma globulin failed to prevent relapses: four occurred, an incidence similar to that in ACTH- or cortisone-treated subjects without conjunct gamma globulin therapy.

3. Oral cortisone produced a rapid initial drop in bilirubinemia but failed to influence significantly the total duration of illness except in two patients receiving therapy over a three-week period.

4. Even under ideal circumstances cortisone does not shorten the duration of disease sufficiently, in our opinion, to run the increased risk of relapse, and thus is not recommended for the routine treatment of viral hepatitis. It may have a place in the treatment of severe and progressive viral hepatitis and certainly deserves further trial in fulminant hepatitis with coma.

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Seminar on Carbohydrate Metabolism

Experimental Diabetes and Its Relation to Diabetes Mellitus

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"There is, however, a Nemesis which waits upon those who deliberately avoid avenues of knowledge." (Alfred North Whitehead, "Science and the Modern World.")

Experimental diabetes refers to the production of hyperglycemia, glycosuria and faulty carbohydrate metabolism in animals. This approach to the problems of diabetes has made possible the discovery of insulin and has enabled physicians to recognize phenomena related to diabetes mellitus which could not have been as readily revealed by the study of man. A few such phenomena will be chosen for comment. Some of these, such as insulin, were formerly hypotheses but have now been demonstrated by subsequent investigation; others provide the working hypotheses of today. The subject of experimental diabetes has been well reviewed by Warren and Le Compte[1] whose chapter provides much of the background for these comments.

Established Phenomena (Initially Mere Possibilities)

Insulin. The existence in the pancreas of a factor which controls carbohydrate metabolism was clearly demonstrated by von Mering and Minkowski in 1889. Thirty-three years later this long recognized possibility was realized in the discovery of insulin by Banting and Best. The fulfillment of this early possibility is cited before considering the problems of today.

Overproduction. In the overproduction theory of diabetes[2] it was assumed that there was no diminution of the utilization of blood sugar by the tissues but that the supply of sugar to the blood from the liver was so excessive that continued normal utilization could no longer dispose of it. Early students of diabetes recognized the possibility that all or part of the picture of diabetes might be due to the excessive catabolism of protein and fat. In both normal and depancreatized animals the early studies on hepatectomy and the modern investigations on the effects of hypophysectomy or adrenalectomy have shown that the secretion of glucose into the blood and the excretion of nitrogen are reduced. When Houssay removed the pancreas in hypophysectomized dogs and observed that the excretion of glucose, nitrogen and ketones were less than the amounts expected after pancreatectomy, he showed that excessive gluconeogenesis (overproduction) was a component of pancreatic diabetes. Later, Young produced diabetes by administration of pituitary extract and left no doubt as to the importance of the anterior pituitary in diabetes.

These discoveries still leave many problems for the future. How frequently does an excessive secretion of pituitary or adrenal hormones act to cause or to aggravate human diabetes? Stress of any kind accelerates gluconeogenesis which is at least in part due to excessive activity of the adrenal cortex.[3–5] The exacerbations of diabetes which accompany infection and certain other diseases are better understood because we now know something about the mechanism of overproduction.

Before the days of antibiotics, when the pancreas was removed the incisions often developed rapidly progressive abscesses. After pancreatectomy in hypophysectomized animals such abscesses healed completely, even in the total absence of insulin and in the presence of hyperglycemia. However, in the animal which had undergone two operations (Houssay) there is no exaggerated breakdown of protein and fat and there is no ketonuria. Since protein and fat breakdown usually occur together, the avoidance of marked ketonuria in man after surgery

might serve as a simple guide in controlling catabolism sufficiently to permit good healing.

Underutilization. From early observations it was postulated that all or some of the phenomena of diabetes might be due to failure to utilize glucose in the normal manner. Recent studies with isotopically labelled substances have resolved this problem as far as the overall or net metabolism of glucose is concerned.[6,7] After total pancreatectomy there is impaired utilization of glucose in the dog[7] and this has been confirmed in man.[8] This impaired utilization is not complete and, in fact, does not differ greatly in degree from that of the fasted normal animal, yet it represents only a small proportion of the utilization of glucose by the normal fed animal. When insulin alone is deficient, the oxidation of glucose and fat synthesis seem to be the metabolic pathways which are particularly affected, but information about these individual pathways requires further study.

In any case the deficiency of insulin is accompanied both by underutilization of glucose and by exaggerated catabolism in varying degrees. To this extent both underutilization and overproduction have been clarified by later observations.

POSSIBILITIES INFLUENCING CURRENT THINKING

Pathways of Carbohydrate Metabolism. In an earlier article of this series Stetten and Topper[9] outlined the knowledge of the pathways of carbohydrate metabolism. Experimental diabetes has contributed much to our knowledge of the biochemistry of carbohydrate, fat and protein.

Mechanism of the Action of Insulin. This is one of the major problems now before us and is reviewed in this series by Stadie.[10] Experimental diabetes continues to be one of the essential methods of the investigator of insulin and it makes possible the broad physiologic picture of diabetes into which the exact information about the action of insulin will eventually be fitted.

The Islands of Langerhans. In 1938 Warren listed toxic injury of the islands of Langerhans as a possible cause of diabetes, although no such cause was known at that time. When Dunn, Sheehan and McLetchie[11] in 1943 described the injury of the islands produced by alloxan, this form of experimental diabetes gave the first factual support to the possibility of toxic injury. Since 1943 other substances which damage the islets have been described.[12] None of these has been identified as a cause of diabetes in man. On hydrolysis of human urine Paley et al.[13] found oxomalonic acid which might have come from alloxan although its exact source has not been ascertained.

Heredity in Diabetes. An increased incidence of the disease in the families of diabetics has brought about recognition of the heredity factor. This and related observations have been reviewed by Joslin et al.[14] Cole, Harned and Keeler[15] found that inheritance in rats of a decreased tolerance for glucose was not a simple recessive characteristic. More recently the "hereditary, obese-hyperglycemic syndrome" in mice has been studied by Mayer.[16] In these mice the islands of Langerhans are increased in size and number; the mice are resistant to insulin and unduly sensitive to the diabetogenic action of growth hormone. Human heredity is so complex that it is often difficult to distinguish genetic, developmental and environmental factors. The breeding of diabetic strains of rats and mice indicates that a genetic factor in diabetes is possible. Knowledge of the various ways in which the disorder is inherited in animals may help in the examination of the problem in man.

Obesity and Diabetes. The regulation of appetite and the various causes and types of experimental obesity have been reviewed by Mayer.[16] The hereditary diabetes of animals already referred to[15,16] is associated with obesity. This condition differs from the situation in the juvenile diabetic who is rarely obese but agrees with the association of events in elderly diabetics. In addition, differences have been observed between the various types of experimental obesity. Marshall and Mayer[17] report that "The food intake of gold thioglucose obese mice is 75–100 per cent greater than that of non-obese animals, the oxygen consumption is greater than that of non-obese animals and the spontaneous physical activity of gold thioglucose mice is similar to that of non-obese animals. These findings contrast with results obtained from genetically obese mice, which are characterized by slight relative hyperphagia, low oxygen consumption, and depressed physical activity. This comparison emphasizes the multiple etiology of obesity." Such metabolic differences in obesity should be thought-provoking to physicians.

Undernutrition in Treatment. Allen[18] on both experimental and clinical grounds emphasized the earlier observations on the value of undernutrition in the control of many of the mani-

festations of diabetes. This is still recognized. Although much has been written about the relation of the vascular complications to the control of the metabolic disorder, there is little information about the influence which the control of obesity might have on these complications. Such work requires many years of clinical observation and is not likely to be solved in the laboratory. Even so, experimental diabetes gives some food for thought. Duff and McMillan[19] found less experimental atherosclerosis in diabetic than in normal rabbits. They said, "gain or loss of body weight during the (cholesterol) feeding period exercised no determining influence." In contrast Firstbrook[20] found a high net correlation between relative weight gain and the severity of experimental atherosclerosis.

Dublin and Marks[21] have reviewed the mortality of obese persons and have said that "A feature of the study is the experience of those overweights who brought down their weight. . . . This is perhaps the best evidence to date that there is long range benefit from weight reduction." The extent to which this improved mortality was due to the prevention of vascular damage was not determined and Liebow et al.[22] concluded that obesity did not influence arteriosclerotic heart disease in diabetes. The specific diabetic changes such as retinopathy and glomerulosclerosis do not develop in obese persons without diabetes; these changes develop in many thin diabetics, but the possibility that control of obesity might be a useful adjunct in some situations still seems to deserve study.

Appraisal of the Severity of Diabetes. Some knowledge of the fundamental observations in the experimental laboratory is required. Although the experimental facts concerned with this appraisal are not new, their meaning is often overlooked by physicians. Various criteria of the severity of diabetes are needed, depending upon the circumstances. Figure 1 graphically represents some of the measurements of the metabolic disturbance in animal and man.

In very mild diabetes only the abnormality of the glucose tolerance test will determine the presence of the disorder. There is no more accurate quantitation at this stage. In a few mild diabetics the blood sugar, under standard conditions such as fasting overnight, may range between normal and the renal threshold of 180 mg. per 100 ml. In these few persons the level of blood sugar probably records differences in the severity of diabetes. When the blood sugar exceeds the renal threshold, it ceases to give information about the severity of the disease. Experimentally pancreatectomy has been combined with administration of phlorhizin.[23] Under these conditions the blood sugar of the depancreatized animals remained at 70 to 80 mg. per 100 ml. but the amount of sugar in the urine, the survival time and all other manifestations of diabetes were unaltered. In Figure 1 the schematic daily blood sugars of moderate and severe (depancreatized) diabetes are merely values above the renal threshold.

The very high values of blood sugar encountered in diabetic coma in animals or man are usually associated with circulatory shock in which the kidney can no longer clear the blood of glucose in its usual fashion. In phlorhizinized-depancreatized cats[23] the blood sugar rose to high levels (for example, 700 mg./100 ml.) before death, and in the series of patients reported by Martin and Wertman[24] all of those with blood sugar levels above 800 mg./100 ml. were in shock. The author regards a very high blood sugar in diabetic acidosis as a sign or warning of circulatory shock.

About 50 per cent of diabetics on a maintenance diet need insulin to control glycosuria. On the chart this group is called MODERATE in severity even though it extends to the point at which 100 per cent of dietary glucose is excreted. In such diabetics (animal or human) the proportion (that is, per cent) of dietary carbohydrate which is excreted in the urine provides a good index of the severity of the disorder. For simplicity one may use all of the dietary carbohydrate and half of the protein of the diet in calculating the so-called available glucose. (The exact D:N ratio is uncertain but a commonly observed factor of this order of magnitude serves the purpose.) The proportion of the available carbohydrate excreted remains fairly constant over a wide range of dietary intake. Dohan et al.[25] described this in a study of pituitary diabetes in dogs and Sturtevant and Fuller[26] have emphasized its usefulness in alloxan diabetic rats.

When a physician is asked, "How severely diabetic is Mrs. X?" he will usually reply, "Severe. She takes 70 units of insulin daily." The dose of insulin needed to control diabetes on a maintenance diet is a useful criterion of the severity of the disease, but it is only one criterion. In Figure 1 the daily dose of insulin is charted as a broken line which parallels the severity of the

disease as measured by the amount of glucose excreted. The end of this line does not touch the baseline because insulin will probably not be used in mild diabetes.

Both the dose of insulin and the proportion of dietary glucose excreted are subject to fairly parallel lines of the present drawing. Nevertheless when gross complicating factors are excluded both of these measurements afford an approximate but serviceable gradation of this segment of the diabetic state.

The most severe diabetes is that due to total

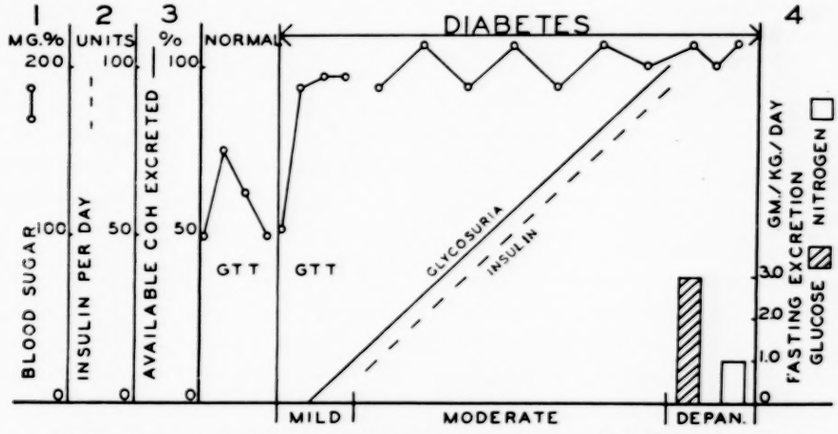

FIG. 1. Four measures of the severity of diabetes (schematic). The units of measurement are numbered 1 to 4 at the top of the chart. The purpose of this diagram is to emphasize the fact that different criteria of the severity of diabetes are required for MILD, MODERATE and total pancreatic (DEPAN.) diabetes. This may be further emphasized by noting what is not charted. Thus the normal person has no glycosuria and needs no insulin administered; the glucose tolerance test (G.T.T.) gives normal results. In mild diabetes the G.T.T. gives abnormal results and glycosuria may or may not be present; hence the solid line that shows the proportion of dietary glucose excreted does not include all of the zone marked MILD. Insulin is not needed in mild diabetes so that the broken line begins still further up the scale of glycosuria. A given point on the parallel lines for glucose excretion and insulin dosage will be raised by infection or lowered by adrenal insufficiency, to cite two examples only.

The zone of MODERATE diabetes is here extended to the point where 100 per cent of available glucose is excreted. In dogs ketonuria appears when 80 to 90 per cent of the available glucose is excreted. Ketonuria is omitted because its appearance and amount are even more irregular than the crude quantification of the proportion of dietary glucose excreted or the dose of insulin. In moderately severe diabetes the level of blood sugar is represented as being constantly above the renal threshold. Hence the blood sugar tells nothing of the severity of diabetes, once it exceeds the renal threshold. The very high levels of blood sugar which are observed in diabetic coma are the result of circulatory-renal failure (shock) and are a secondary effect of severe diabetes but they do not measure the metabolic defect. Therefore, such figures have been omitted. Finally, severe diabetes in the human usually has little or no glycosuria during fasting (compare with Table 1) so that the excretion of glucose and nitrogen during fasting is not charted except under DEPAN. Due to the defective absorption of food after pancreatectomy the maintenance requirement for insulin cannot be compared to that for diabetes in animals or men whose digestive enzymes are functioning. For this reason insulin dosage is not charted in the DEPAN. zone.

wide variations with complicating circumstances. One of the most important of these is infection, which will increase glycosuria and insulin requirements. In contrast the reduced glycosuria of the Houssay animal or of the patient with adrenal insufficiency and diabetes would be charted here by lowering a given point on both pancreatectomy. (Fig. 1.) This is so because of the absence of insulin and the violent metabolic reaction thereto. Under these conditions the dog and cat excrete about 3.0 gm. of glucose and 1.0 gm. nitrogen per kg. per day during fasting, so these amounts have been used in the diagram. Since most diabetic patients become sugar-free

on fasting, such units of measurement are not used elsewhere in Figure 1.

Table 1 reviews some frequently cited data about the response to pancreatectomy in different species as measured by the fasting metabolism. The observations of Geyelin and Du Bois[27]

TABLE 1
FASTING METABOLISM IN DIABETES

Species or Subject	Urine				D:N	Survival (days)
	Glucose (gm./kg./day)		Nitrogen (gm./kg./day)			
	Normal	Depancreatized	Normal	Depancreatized		
Cat	0	3.2	0.7	1.4	2.3	4
Dog	0	2.8	0.5	1.0	2.8	10
Goat	0	0.12	0.13	0.38	0.3	44
*C. K.[27]						
Dec. 1915	0	1.3*	.....	0.41*	3.1	..
Jan. 1916	0	0	0.17†	0.17*	0	..
F. W.[29]	0	....	.....	.....	2.4	6

* Severely diabetic, not depancreatized.
† Normal man; average of first nine days of fasting (calculated from F. G. enedict[30]).

on a severely diabetic man have been added for comparison. In December 1915, this patient (C. K.) had mild acidosis from which he recovered as a result of undernutrition and drainage of a carbuncle; this treatment would not save the depancreatized dog. The increased excretion of nitrogen during this period ("higher than any hitherto observed in man") resembles the increase seen in animals after pancreatectomy, but the patient's spontaneous recovery makes one hesitate to assume that these figures represent an absence of insulin. He ultimately tolerated 160 gm. of carbohydrate and 3,500 calories daily. According to Table 1, the wasting of protein (nitrogen) during fasting is a finding common to all kinds of depancreatized animals. This is so regardless of variations in the amount of glycosuria, the period of survival, the presence or absence of ketonuria and other factors.

In connection with the problem of measuring the severity of diabetes, two references may be cited. First, Lusk[28] reported a man with mild diabetes who could take 40 gm. of carbohydrate daily without glycosuria. "He became completely diabetic with a urinary D:N of 3.84:1 on the second day after administering a diet containing considerable quantities of protein and fat. Subsequently, virtual starvation led in 60 hours to the disappearance of sugar in the urine. It was thus proved that a potential diabetic could be transformed into a completely diabetic individual. . . . " The fasting D:N of patient C. K. in January, 1916 (Table 1) and the contrasting D:N of Ricketts'[29] patient suggest that most of the patients reported by Lusk were not completely diabetic. What indeed is "complete" or "total" diabetes? It is the fulfillment of some criterion adopted by the particular investigator. The author has tried to avoid such terms and can only say that the most severe form of diabetes appears to be that which meets appropriate criteria of the totally depancreatized animal of the same species. In a study of dogs with pituitary diabetes,[25] three animals were encountered in which the fasting excretion of glucose and nitrogen equaled that of depancreatized dogs, although in pituitary diabetic dogs the pancreas was surgically untouched.

Second, Mirsky[31,32] has ably used the fasting metabolism to elucidate the fact that diabetes of the depancreatized dog is more severe than that of the alloxanized dog although the latter requires more insulin when fed. The desirability of obtaining information of this sort about man is apparent from the references already cited and from the fact that these spaces for depancreatized man remain blank in Table 1.

In Figure 1 insulin dosage refers to the amount per day which is needed to control diabetes on a maintenance diet. The insulin requirements of the depancreatized man, often about 40 units daily, cannot be compared to the requirements of other diabetes because of the variable but grossly defective absorption of protein and fat after this operation, even when the best available exocrine therapy is given.[29] Most uncomplicated diabetics need no insulin during fasting if one may judge from the old records and, as the author knows of no data on the insulin requirement of the fasting depancreatized man, no figures are presented for comparison. Depancreatized dogs, which needed 40 units of insulin daily when fed, were well controlled on four units daily during fasting.

Another type of study on food intake and insulin requirement has been performed by Singh.[33] This included six patients with severe diabetes who originally required 80 to 120 units of insulin daily. After six weeks on low-fat optimal calorie diets containing 20 to 30 gm.

of fat these severely diabetic patients could be controlled on 20 to 40 units daily. Since the absolute amount of fat fed to these patients coincides in a general way with the amount absorbed by depancreatized men (compare studies cited in Ref. 29), the achievement of a similar insulin dose in both instances seems noteworthy. Such experiences prepare one to find a lowered insulin requirement with the defective food absorption which follows pancreatectomy. The author has not yet heard of a depancreatized person becoming obese.

Only passing reference will be made to certain other criteria of the severity of diabetes. The period of survival after pancreatectomy is listed in Table 1 and the only clinical observation[29] makes human pancreatic diabetes comparable to that of the dog or cat in this respect. Another criterion is the rate of weight loss after pancreatectomy. This is greatly influenced by the catabolic reaction to the removal of insulin, as shown by simple laboratory findings. Thus depancreatized cats lose 300 to 400 gm. in the four days of survival; depancreatized-hypophysectomized cats lose the same amount of weight in two months. Both lose weight because they lack insulin, but when the pituitary and adrenal cortical catabolic hormones are in action this process is violently accelerated. In a slightly different way the same thing has been observed in man. Thus a fasting man[28] lost an average of 0.57 kg. per day during the first ten days. Patient F. W.[29] lost 1.5 kg. daily in the first three days of a period without insulin, or almost three times the rate of the fasting normal man.

In conclusion: (1) both experimental diabetes and clinical experience make it clear that multiple criteria of the severity of diabetes are required under different conditions; (2) choice of these criteria has been difficult in the past and may well be revised by future experience; and (3) there is basic information still to be learned about human diabetes by applying experimental criteria to depancreatized man.

Table 1 illustrates the response to pancreatectomy in different species. Other species differences exist, three of which will be mentioned.

1. The response to so-called diabetogenic hormones varies widely with the species. This is presumably an extra-pancreatic difference in the animals and may be illustrated by the following well known laboratory findings.

The rat develops hyperglycemia and glycosuria when treated with corticotrophin or cortisone, both of which are ineffective in dogs and cats. On the other hand pituitary growth hormone is ineffective in rats but is a powerful diabetogenic agent in cats and dogs. Unpublished observations (Lukens) on the production of diabetes in cats with some new synthetic cortisone derivatives seem to indicate that this is the result of the degree or intensity of hormonal action as well as of the susceptibility of a particular species.

2. When hyperglycemia and glycosuria are produced by any means, the behavior of the islands of Langerhans differs greatly in different species. In the rat the islands do not suffer any appreciable degenerative changes. Even when they are overwhelmed by a diabetogenic hormone the diabetes ceases when the hormone is discontinued. The rat also has considerable capacity for islet hyperplasia. Contrariwise, in the cat and dog partial pancreatectomy, pituitary extract (growth hormone) or massive injections of glucose lead to degenerative changes and atrophy of the beta cells of the islands, so that permanent diabetes may ensue. Relatively little capacity for islet hyperplasia exists in these animals.

3. Even in the same species the islands of Langerhans may behave differently at different times. The normal cat given massive injections of glucose develops degranulation, hydropic degeneration and atrophy of the islands. After the administered glucose is discontinued the animal may have mild diabetes for an indefinite period. At this time the same degree of hyperglycemia and glycosuria leads to no progression in the severity of the diabetes. No explanation for these observations exists but the facts muct be reckoned with as one attempts to learn more about the islands of Langerhans in human diabetes.

The different behavior of the islands in the rat and the dog cannot be foretold by their essentially similar appearance histologically. Little is known of the behavior of the islands in man and such laboratory experiences should alert us to look for these and other possibilities in the islands in human subjects.

Transient Causative Factors. Experimental diabetes has demonstrated that a causative agent of diabetes may have a transient existence and may not be observed in a diabetic animal studied at a later date. We do not know the extent to which this may apply to human diabetes and it has been said that the cause or causes of diabetes

may have to be determined before the person studied actually becomes diabetic.

Experimental studies of the influence of diet on the islands of Langerhans[34] combined with further observations on obesity as a precursor of diabetes in man[14,21] should yield more knowledge of the diabetic state. The development of diabetes years after women have been delivered of large babies[35] may provide another source of information about the conditions that precede the overt metabolic disorder. Meanwhile the facts that (1) no alloxan is present five minutes or so after its injection and (2) no excess of pituitary activity is demonstrable after the injections of pituitary extract have ceased, make every clinical investigator aware that similar events might occur in man.

Time Factors in the Cause of Diabetes. The cause or causes of diabetes in man may require variable periods of time to produce the disease. Alloxan damages the islands in a matter of minutes, pituitary extract in four to six weeks. Both the diabetogenic agent and the behavior of the islands will be concerned with the time factor in the cause of diabetes. To complete the picture one need only note that in man obesity is commonly present for twenty years or more before diabetes appears. Certainly no time limit of the causative agent has been set for man.

Vascular Complications. The so-called vascular complications, especially those of the retina and kidney, which are fairly specific lesions of diabetes, may become the subject of laboratory study. Two groups of investigators[36,37] have reported the development of renal lesions in rats within a few months after they had been made diabetic, and Janes and Bounds[38] have demonstrated an abnormality of the retinal capillaries in diabetic rats. If lesions in any way comparable to those which usually take ten to fifteen years to develop in man can be produced in less time and so studied in the laboratory, it should be a forward step in the attack on these complications. Dr. E. H. Rynearson[39] reported that one patient depancreatized for intractable hypoglycemia developed diabetic retinopathy twelve years later. Such information accruing simultaneously from laboratory and clinic should stimulate future investigation.

SUMMARY

A few selected possibilities about the nature, causation and measurement of experimental diabetes have been outlined. A modest comprehension of these experimental experiences should be of some help in the management and study of the disease in man.

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Case Reports

Treatment of Scleroderma, Sclerodactylia and Calcinosis by Chelation (EDTA)*

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THIS is a report of our observations concerning the use of a chelating agent, ethylenediamine tetracetic acid (EDTA), in a patient with scleroderma, sclerodactylia, calcinosis and, possibly, rheumatoid arthritis. Chelating agents have been used medically in cases of lead poisoning;[1] for the removal of urinary calculi;[2] to dissolve calcific opacities in the eye;[3] and to dissolve metastatic calcium deposits in the kidney.[4]

EDTA combines with metal ions to form cyclic complexes which are water-soluble and virtually undissociated.[5] When EDTA is administered rapidly as the sodium salt a rapid lowering of the serum calcium levels results. This is due to the strong affinity of the sodium salt for calcium ions, forming a complex by chelation. When given rapidly in large doses EDTA may cause hypocalcemic tetany. When given slowly homeostatic mechanisms come into play which tend to maintain serum calcium levels by mobilization of calcium from skeletal reserve stores.[6]

Sodium versenate causes an excess of urinary calcium to be excreted. This excess, expressed in per cent of the expected calcium binding power of this agent (1 gm. di-Na-EDTA complexes with 108 mg. calcium), ranges from 63 to 88 per cent of the injected dose.[7]

CASE REPORT

A forty-five year old woman was admitted to the Greenpoint Hospital on February 16, 1954. The patient complained of muscular aches, joint pains and swelling; weakness and inability to walk, stand or use her hands; the presence of hard nodules on her fingers, wrists, elbows and knees; flexion deformities of her fingers, hands, elbows and knees; and a feeling of constriction in her chest. The onset was in 1944 with intermittent pain and swelling of both knees. In spite of treatment by local physicians her illness progressed until 1949 at which time both hands and fingers were involved. It was noted in 1949 that irregular, hard nodules were forming around the fingers and wrists of both hands and at the left elbow. The weakness increased in both hands and by 1951 she was unable to use them.

In 1951 she was given 100 mg. of cortisone daily and the pain and swelling subsided. In November, 1951, after eleven months on this dosage, she manifested psychic symptoms. Medication was stopped and she was admitted to the Kings County Hospital for treatment of cortisone psychosis. She was discharged after six weeks and then was given seven electric shock treatments by a private physician.

Her pains returned thereafter and in September, 1952, she was started on gold therapy. This was given twice a week for six weeks and then discontinued because of lack of improvement. She continued to grow weaker and the pain, swelling and stiffness in the joints increased. In November, 1952, she was admitted to the Long Island College Hospital because of these complaints. Here she received butazolidin,® 300 mg. per day. This relieved her complaints completely but after nine days of butazolidin therapy exfoliative dermatitis, ulcerative stomatitis and mild leukopenia developed. The drug was stopped but the complications did not clear up. While in the hospital a biopsy of skin nodules showed "calcified nodules of subcutis"; a biopsy of skin from the chest wall was reported

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as follows: "The epidermis is relatively thin but shows a surface layer of keratin. The rete pegs are shortened and show blunted ends. The dermis is fibrotic and shows alteration in the pattern of distribution of collagen bundles. In some regions these form solid hyalinized sheets.

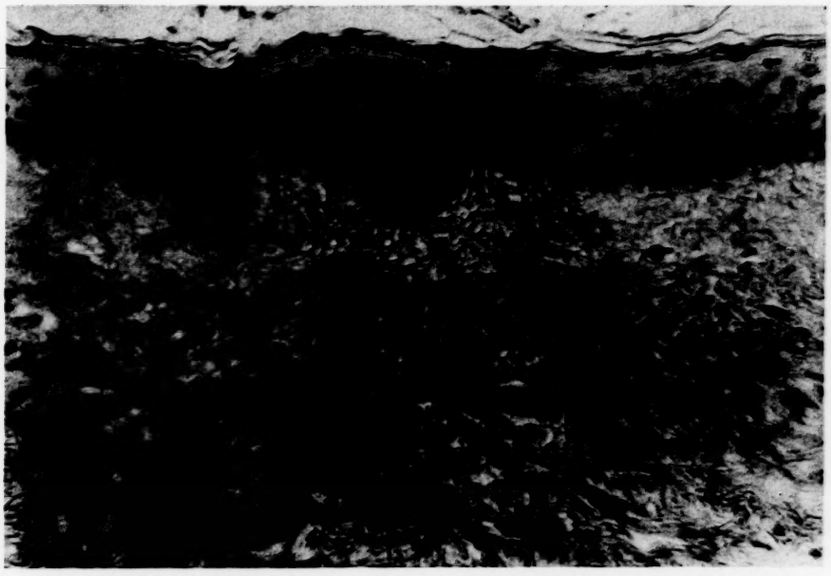

FIG. 1. Skin from right chest wall before treatment. See text for description. (Original magnification × 400.)

The secondary cutaneous appendages are relatively reduced and there are foci of lymphocytic infiltration about capillaries in the dermis. Diagnosis—Scleroderma." (Fig. 1.) The patient was discharged December 16, 1952.

Her skin condition did not improve while at home and she entered the Greenpoint Hospital on January 16, 1953, and was treated by the Dermatology Service. After eight weeks of treatment the skin improved and she was discharged. While at home she took about 50 gr. of aspirin daily for her pain, stiffness and swollen joints.

Family history revealed that the patient's mother had been ill for many years with rheumatoid arthritis.

Physical examination revealed a cooperative, intelligent, anxious female. The patient could not turn or move in bed without assistance. The face was smooth, the features were rigid and without expression. The skin over the face, chest, hands and fingers was "porcelain white," hard, shiny and without elasticity. The finger tips were clubbed and the fingers were held in flexion. (Fig. 2.) There was swelling of the proximal and distal interphalangeal joints. Hard, irregular, nodular masses were present on the fingers of both hands and on the left elbow and both knees. (Fig. 3.) Pressure on these nodules discharged an amorphous, sticky material. Motion in the right wrist was greatly limited in all directions. The left wrist motion was slightly limited due to muscle spasm. Both knees and elbows were swollen, hot and painful. The left knee extended to 130°, flexed to 90°; the right knee extended to 140°, flexed to 90°. Both elbows extended to 140°, flexed to 45°. No hair was present on the fingers, hands, forearms or legs. The chest expansion was from 36½ to 37 inches. The blood pressure was 125/70.

On February 16, 1954, the following tests were performed and the laboratory data were as follows: The urine was negative. The Sulkowitch tests were 1+ to 2+. Blood count: hemoglobin 9.3 gm. per cent, red cell count 3,110 million, white cell count 6.25 thousand. The blood urea nitrogen was 16 mg. per cent; sugar 65 mg. per cent; serum calcium 9 mg. per cent; phosphorus 3.2 mg. per cent; alkaline phosphatase 2.8 Bodansky units. The basal metabolic rate was +40 and on February 27th, +20. The discharge from the finger was found to contain a "moderate amount of calcium." Biopsy of skin from right chest wall showed "partial atrophy of epidermis and appendages. Areas of edema and dense collagenous tissue in the corium with mild chronic focal inflammation." Biopsy of skin and subcutaneous tissue

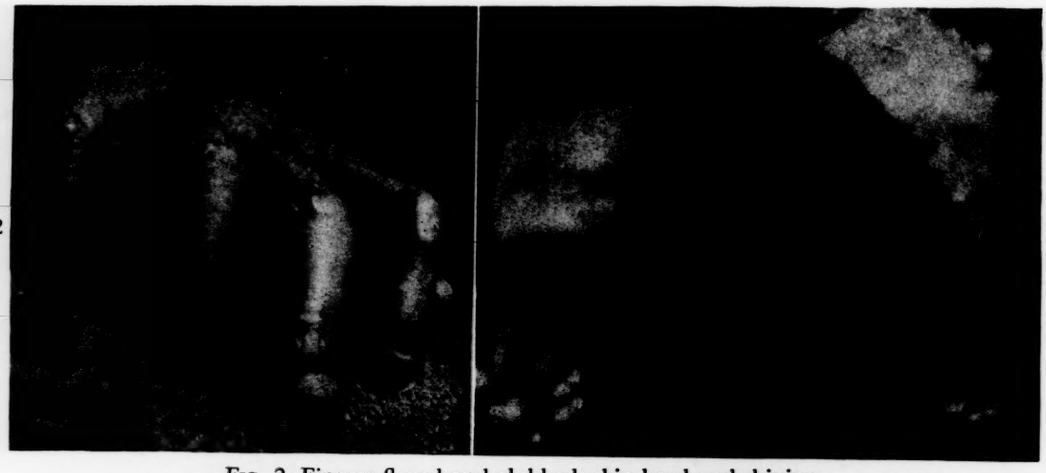

FIG. 2. Fingers flexed and clubbed; skin hard and shining.
FIG. 3. Left elbow; hard, irregular, nodular areas present.

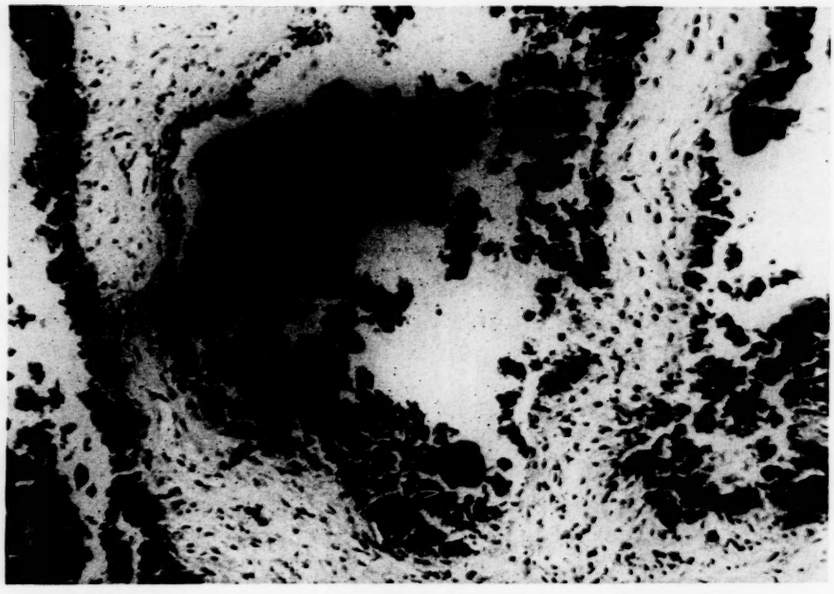

FIG. 4. Skin and subcutaneous tissue from finger before treatment. There is one large and several miniature foci of calcification and slight diffuse and focal chronic inflammation. (Original magnification × 400.)

from the right middle finger showed "one large and several miniature foci of calcification and slight diffuse and focal chronic inflammation." (Fig. 4.)

Blood tests were made on May 4th to check for evidence of rheumatoid arthritis and rheumatic fever. The results were: differential sensitized sheep cell titre = 1:32 (positive); hemolytic streptococcus agglutination, negative; C-reactive protein, negative.

The admission diagnosis was scleroderma, sclerodactylia, calcinosis and possible rheumatoid arthritis.

On May 1, 1954, it was decided to make a trial test of chelation for removal of the calcium deposits. The patient was placed on a diet containing 1.2 gm. of calcium daily. Urinary calcium and blood serum calcium determinations were made. On May 10, 1954, chelation therapy was begun and continued each day for one week. Sodium versenate, supplied by the Riker Drug Co. of Los Angeles, California, was used. This is the sodium salt of ethylene diamine tetra-acetic acid, 20 per cent. (Each 5 cc. of solution at approximate neutrality contains 1.0 gm. in water for injection.) Three grams of

FIG. 5. Scars over proximal interphalangeal joint of left middle finger and right little finger. Shortening of left middle finger.

the drug were dissolved in 500 cc. of 5 per cent dextrose in water. About three and a half hours were needed to complete the intravenous infusion. Twenty-four-hour urine samples were collected and analyzed for calcium content. The calcium determinations were made at two laboratories. At the hospital Mr. Baber used the method described by Hawk, Summerson and Oser.[8] At the Gardner Laboratory Mr. Halpert used the method of Shohl and Pedley.[9] Prior to therapy the patient's urinary calcium excretion averaged 165 mg. per twenty-four hours. During therapy it averaged 293 mg. per twenty-four hours, fluctuating between 210 and 391 mg. The blood serum calcium varied as follows: May 3rd 10.8 mg. per cent; 11th, 7.5 mg. per cent; 12th, 8.3 mg. per cent; 13th, 11.5 mg. per cent; 17th, 9.8 mg. per cent. That part of the calcium bound by the chelating agent was not ashed and could not be determined.

At no time was there any evidence of toxicity, tetany or convulsions. At the beginning of each infusion the patient experienced a mild feeling of heat starting in the arm receiving the medication. This would gradually spread over the entire body and last about two hours after the infusion. The temperature remained normal. There was no flushing or sweating. After the infusion the patient ate and continued her activities. She received physiotherapy, massage and active, passive and resistive exercises regularly. After one week of infusions her chest felt "as if a weight were removed from it." She could breathe more easily. Later she felt "as though all the muscles were loosening up." Chest expansion increased to 37½ inches. Her entire skin felt softer and had lost the porcelain-white look, at times showing a faint pinkish tone. She felt much stronger and moved about in bed with less discomfort than on admission. The aches, pains and joint swelling had decreased. The nodular areas, x-rays of the extremities and flexion deformities were practically unchanged. She was discharged May 22, 1954, improved.

She remained at home until August 25, 1954. During this three-month interval she got around the house in a wheelchair, had pain occasionally, felt stronger and could breathe without difficulty. She noted for the first time that hair was growing on her arms. On July 1, 1954, the proximal interphalangeal joint of the third left finger became red, hot and swollen. The skin broke down, discharging a watery, sticky material. This continued until August 15th at which time two small pieces of bone were discharged. Shortly thereafter drainage stopped and the fistula closed. (Fig. 5.) Aspirin, 40 to 50 gr. daily, kept her comfortable.

She was readmitted on August 25, 1954, to the Arthritis Service, Greenpoint Hospital, because she felt that versene was the first drug that had helped her and desired to give it a longer trial.

Readmission examination was performed on August 25, 1954. The patient's outlook was more cheerful and optimistic. Abduction, left shoulder, to 150°, right to 165°; left elbow

extends to 145°, flexion to 45°; right elbow extension 145°, flexion 45°. The right wrist was immobile, the left was freely movable. There was some wasting of interossei muscles of both hands. The little finger of the right hand was held in flexion. There was clubbing of the right thumb, index and middle fingers. Calcium nodules were felt in the thumb and index fingers. There was clubbing of the left thumb and index fingers. Calcium deposits were present in the left thumb, index, middle and ring fingers. A healed scar was present on the proximal interphalangeal joint of the left middle finger; this finger was ½ inch shorter than on discharge. The function of the hips was good. The left knee extended to 100°, flexed to 90°; the right extended to 130°, flexed to 90°. There were nodules over the left elbow and both knees.

She was placed on a diet containing 1.2 gm. of calcium. After four days of control observations, intravenous medication with the same chelating agent was begun August 30, 1954. This was continued on alternate weeks until October 22, 1954.

Blood chemistry and urine tests were performed and revealed the following laboratory data:

August 25 to October 26, 1954: (1) RBC remained about 3,450,000; hemoglobin, 60 per cent. (2) Urea nitrogen: 17.8 mg. (8/25), 8.3 mg. (9/14), 22.5 mg. (10/27). (3) Bleeding time: 2 min. 10 sec. (9/2), 1 min. 25 sec. (9/13), 5 min. 30 sec. (10/25). (4) Clotting time: 5 min. 45 sec. (9/2), 3 min. 2 sec. (9/13), 6 min. 5 sec. (10/15). (5) Sedimentation rate (Linzenmeier method): varied from 16 min. to 50 min. (6) Total protein: 8.2 gm. (10/5), 9.2 gm. (10/25). Albumin: 2.4 gm. (10/5), 2.7 gm. (10/25). Globulin: 5.8 gm. (10/5), 6.5 gm. (10/25). Inorganic phosphorus was elevated; chlorides were decreased. The other blood findings were normal.

Performance tests were checked and revealed the following information:

September 11, 1954
- Head and neck—Rotation: left, 45°; right, 45° Flexion and extension, good
- Shoulders—Full arm flailing: left, twelve times; right, thirteen times per minute
- Elbows—Supination and pronation: left, all movements good; right, 50 per cent
- Finger tip apposition—Complete
- Fist closure—Right, 30 per cent; left, 0
- Sitting—Flopped into chair and could not rise; could not stand, kneel or squat
- Could not walk, climb, hop or put foot on chair

October 9, 1954
- Head and neck—Rotation, flexion and extension, complete
- Shoulders—Full arm flailing: right and left sixteen times each per minute
- Elbows—Supination and pronation: left, all movements good; right, 50 per cent
- Finger tip apposition—Good
- Fist closure—Right, 40 per cent; left, 20 per cent
- Sitting—Sat without help; did not flop, could rise from chair
- Could stand when wearing shoes; could walk in walker; could not climb, kneel or hop

December 2, 1954
- Shoulders—Full arm flailing: right and left each forty-five times per minute
- Elbows—Supination and pronation: left, all movements good; right, 75 per cent
- Fist closure—Right, 60 per cent; left, 40 per cent

The tests previously cited were repeated during and following treatment. (Tables I and II.)

By September 1st the patient was able to cross her knees for the first time. She felt much stronger. On September 9th the proximal interphalangeal joint of her right fifth finger became red, hot and swollen. The skin broke down, discharging a sticky material. The area healed slowly. (Fig. 5.) On September 10th she began to walk in a walker for the first time in a year. She received codeine and aspirin tablets for pain. By October 7th she felt increasingly stronger and more confident. Her activities of daily living improved steadily. (Table I.) Physiotherapy, exercise and massage were continued. It was noted that a few small nodules had formed on her chin, left elbow, both knees and fingers. By November 15th further subjective and objective improvement was noticeable. A skin consultant on September 21, 1954, found "no evidence of scleroderma." On November 16, 1954, biopsy of skin and subcutaneous tissue from right chest wall showed "The epidermis is not altered. The papillary bodies are better developed than in the section of November 1952, although there are stretches where they are flat

TABLE I

Tests for Activities of Daily Living	Sept. 1, 1954	Nov. 15, 1954
Non-walking Activities		
Bed activities:		
Move from place to place in bed	Poor	Good
Roll to right and then to left side	Poor	Good
Sit erect in bed	0*	Fair
Turn and lie on abdomen	0	Fair
Procure objects from night table	0	Good
Hygiene (toilet activities):		
Comb or brush hair	0	Fair
Brush teeth	0	Good
Shave or put on cosmetics	Fair	Good
Wash hands and face	Fair	Good
Wash extremities	Fair	Good
Manipulate bedpan	0	Good
Adjust clothing for toilet needs	0	Good
Eating activities:		
Cut meat	0	Good
Butter bread	0	Good
Eat with fork	0	Good
Eat with teaspoon, tablespoon	Poor	Good
Drink from glass	Poor	Good
Drink from cup	Poor	Good
Stir coffee, tea, etc.	Poor	Good
Dressing and undressing activities:		
Put on underclothes	0	Fair
Remove underclothes	0	Fair
Put on buttoned shirt (zipper)	0	Fair
Remove buttoned shirt	0	Good
Put on slip-over garment	0	Good
Hand activities:		
Write name and address	0	Fair
Fold letter, place in envelope and seal envelope	0	Fair
Open envelope, remove letter	0	Fair
Use dial telephone	Poor	Good
Turn pages of book	Poor	Good
Wind wrist watch	0	Good
Open and close icebox door	0	Good
Open and close doorlock with key	0	Good
Open and close drawers	Poor	Good
Open and close door hooks	Poor	Good
Pull window shade	Fair	Good
Push door bell	Fair	Good
Use work push buttons	Poor	Good
Work key light switch	Poor	Good
Work pull chair light	Poor	Good
Ring door bell	0	Good
Open and close cabinet lock	0	Good
Turn four-pronged faucet	0	Fair
Turn circular faucet	0	Fair
Open and close medicine chest	0	Fair
Open and close bottle	0	Fair
Open and close safety pin	0	Fair
Strike match	0	Fair
Wheelchair activities:		
Bed to wheelchair	0	Fair
Wheelchair to bed	0	Fair
Propel wheelchair forward 30 feet and stop	Poor	Good
Propel wheelchair backward 30 feet and stop	Poor	Good
Wheelchair to chair	0	Fair
Chair to wheelchair	0	Poor
Elevation activities:		
Bed to erect position	0	Fair
Erect position to bed	0	Good
Wheelchair to erect position	0	Fair
Erect position to wheelchair	0	Fair
Chair to erect position	0	Fair
Erect position to chair	0	Good
Erect position to chair at table	0	Fair
Chair at table to erect position	0	Good
Walking Activities		
Progressing activities:		
Walk forward 30 feet in walker	Poor	Good
Walk backward 30 feet in walker	Poor	Good

* Zero (0) = no activity

and blunted. The subepidermal layer of the dermis is not as compact as in the above mentioned section, although there are stretches where hyaline bands are visible but they are narrow. In its entirety the dermis is much looser and the collagen bundles separated. The sweat glands are large, the basement membranes of the individual units are not conspicuous. The capillaries of the upper corium are far more conspicuous compared with the earlier section." (Fig. 6.) At this time the left elbow became red, hot and swollen. The skin broke down and discharged a pasty material which became hard on exposure to air and crumbled on pressure. Small, hard granules were present in the discharge. A smear of the pus was positive for staphylococci. This infection responded to antibiotics and the elbow decreased in size.

TABLE II
GRIP TESTS

	Right (mm. Hg)	Left (mm. Hg)
Aug. 25, 1954	46	86
Sept. 9, 1954	70	86
Sept. 23, 1954	50	50
Oct. 28, 1954	120	100

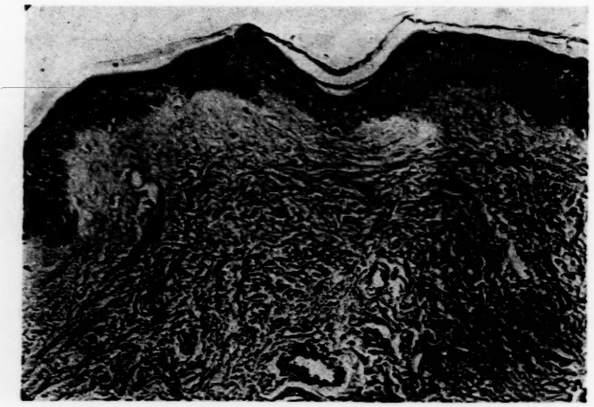

FIG. 6. Skin from right chest wall after treatment. See text for description. (Original magnification × 200.)

Examination on November 19, 1954, revealed a determined, energetic, optimistic individual. She displayed increased agility in getting up in bed, moving on to a chair and then to the walker. The motivation was excellent. The skin of the body and extremities was softer and had increased tone. The face wrinkled when she laughed. The hands and fingers had lost their hard, cold appearance and were softer and wrinkled. Hair was now present on the forearms and legs. Chest expansion was from 36½ inches on expiration to 38 inches on inspiration. The patient was discharged November 20, 1954, very much improved.

On March 18, 1955, follow-up laboratory tests were made to check for possible drug toxicity. The following results were obtained: Sugar, 87 mg. per cent; blood urea nitrogen, 11.9 mg. per cent; sodium, 154 mEq./L.; chlorides, 115 mEq./L.; calcium, 10.3 mg. per cent; and the carbon dioxide combining power, 58 volumes per cent. The total protein was 7.8 gm. per cent (albumin 2.5, globulin 5.3 gm.). Urinalysis: light yellow, clear, acid; specific gravity, 1.004; protein and glucose tests, negative; microscopic examination showed 25 to 30 white blood count and many epithelial cells. The Fishberg test gave results as follows: 8 A.M., 1.011; 9 A.M., 1.006 and 10 A.M., 1.004. The urinary calcium excretion was .067 gm./twenty-four hours. Red blood count was 3.81 million; hemoglobin, 10.8 gm. (70 per cent); color

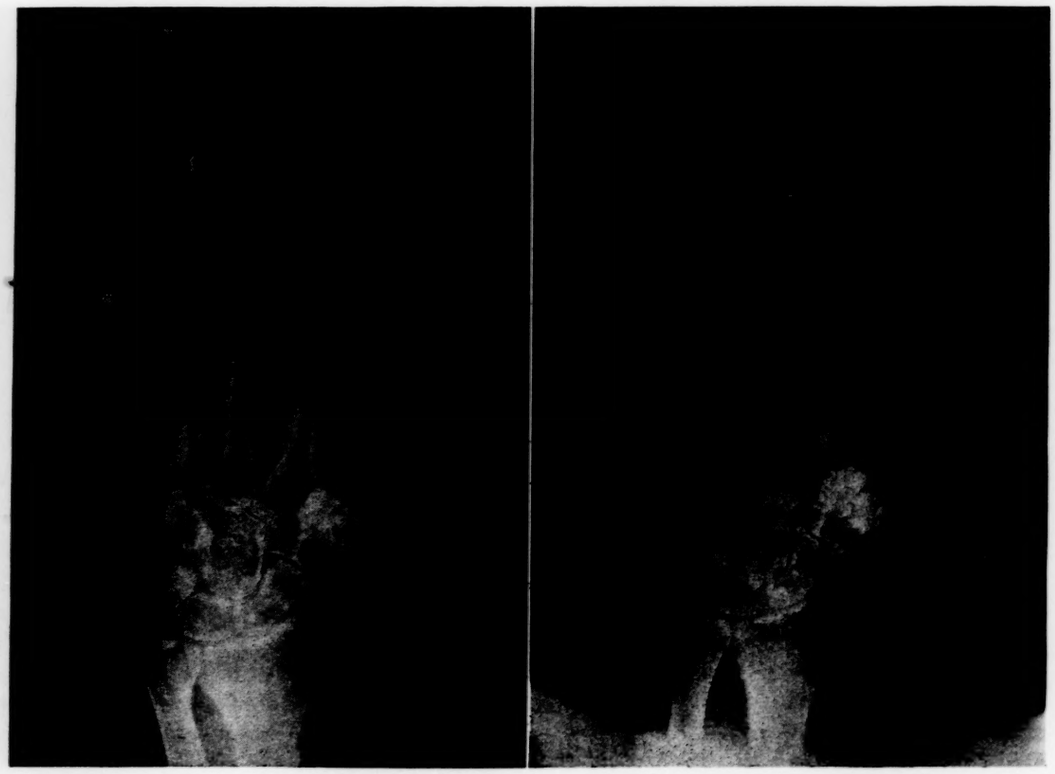

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FIG. 7. May 19, 1954. Left hand before treatment. Diffuse calcification involving the phalanges.

FIG. 8. January 22, 1955. Left hand after treatment. Decrease in calcification most marked in thumb and middle finger.

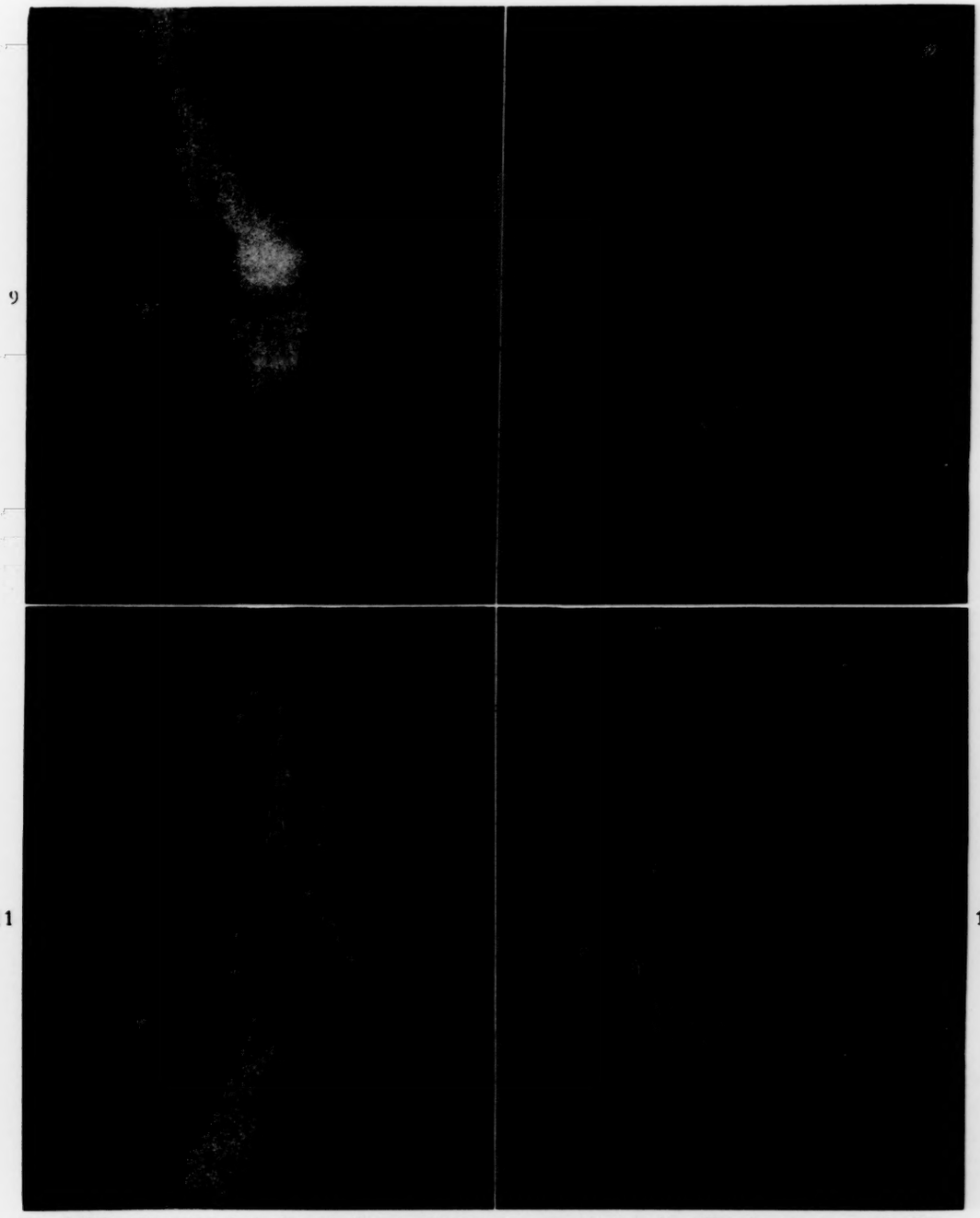

Fig. 9. May 19, 1954. Left elbow before treatment, anteroposterior view. Two large masses of calcium are present.

Fig. 10. August 26, 1954. Left elbow before treatment, lateral view. A large calcific mass present around the joint.

Fig. 11. February 27, 1955. Left elbow after treatment, anteroposterior view. Almost complete disappearance of the calcium deposit seen previously.

Fig. 12. February 27, 1955. Left elbow after treatment, lateral view. Almost complete disappearance of the calcium deposit seen previously.

index, 0.92; white blood count, 7,050; differentials: Schilling cells, segmented, 59 per cent; staff forms, 5 per cent; lymphocytes, 32 per cent; eosinophils, 4 per cent; platelets, 150,000. Prothrombin time was 17.9 seconds and the control was 12 seconds. The first specimen of the urea clearance test was 108 per cent of average normal and the second specimen was 71 per cent of average normal.

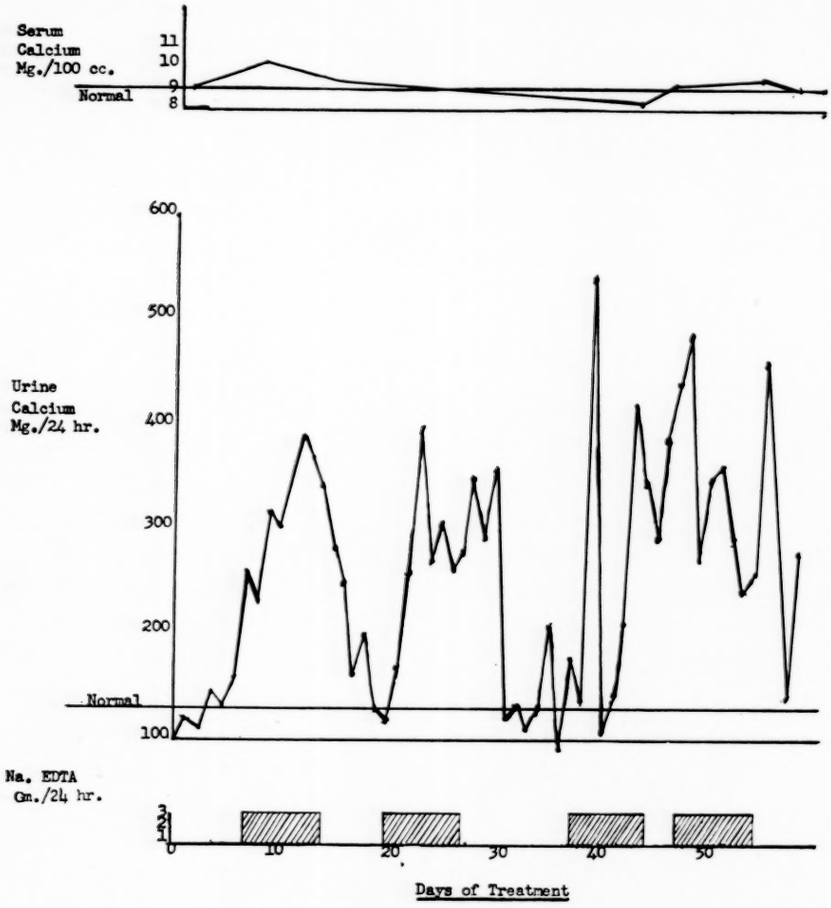

FIG. 13. Response to treatment.

COMMENTS

This patient had a progressive form of arthritis of eleven years' duration. Five years after the onset of her illness she began to show evidence of scleroderma, sclerodactylia and calcinosis. This is a slow, progressive condition with periods of remission and exacerbation. There is no known effective treatment. During her period of observation and treatment in the hospital she was on a fixed diet. This did not affect her calcium deposits.

Since chelation had been used successfully to dissolve urinary calculi, calcific deposits in the eye, and to remove lead from bones, it was thought that it might also remove calcium deposits from the skin and connective tissues.

If the x-rays before and after treatment are compared, marked loss of the calcium deposits is noted. (Figs. 7 to 12.) Table 1 shows distinct improvement in the activities of daily living. This improvement is also shown in her performance tests. Other interesting observations were the softening of the skin and subcutaneous tissues and disappearance of nodules in the left elbow; also, the growth of hair on her forearms and legs. There was a change in the skin biopsy findings. The flexion deformities decreased and joint extension and motion increased.

During treatment there was a definite increase in the output of urinary calcium but no marked change in the level of serum calcium. (Fig. 13.) Bleeding and clotting time decreased; the blood pressure remained unchanged. There was no evidence of toxicity from the drug at any

time during or after medication. An interesting observation is that x-rays first showed evidence of the disappearance of calcium on January 2, 1955, i.e., about four months after the start of intensive therapy.

From the patient's subjective point of view the treatment was of definite benefit. As of March, 1955, the patient felt stronger and had more confidence in herself. She had greater use of her limbs and was able to do many things she could not do previously.

SUMMARY

A patient with typical scleroderma, sclerodactylia, calcinosis and arthritis (rheumatoid?) was treated with a chelating agent, EDTA, given by infusion. Treatment was followed by improvement as indicated by x-ray evidence of marked diminution in the articular and cutaneous metastatic calcific deposits, histologic evidence of regression of the sclerodermatous changes in the skin and return of mobility of the affected joints.

It seems probable that the chelating agent was responsible for remission.

Acknowledgment: The authors wish to express their appreciation to Dr. M. Ziff for his suggestions including the use of the "versenes," and to the Riker Laboratories of Los Angeles, California, for supplies of the sodium versenate.

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Phrenic Paralysis Due to Serum Neuritis

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In routine fluoroscopic examinations of the chest over a period of thirty years, occasional patients have been observed to have paralysis of the diaphragm without satisfactory explanation after careful investigation had excluded the accepted causes of such a finding. In the summer of 1951 the first patient being reported was observed with orthopnea due to phrenic paralysis shortly after a severe episode of tetanus antitoxin serum neuritis. The possibility of this as the source of his trouble was considered, but not being acquainted with any description of phrenic palsy due to serum neuritis, and because he had a vague density in the right upper chest, a tentative diagnosis of Pancoast's tumor was made. Shortly after this the report of Comroe et al.[1] on a similar case appeared and the diagnosis was changed to phrenic paralysis due to serum neuritis. In 1952 another instance of paralysis of the diaphragm was noted and close questioning revealed that the patient had had serum neuritis. A third patient was seen in 1953. He had pneumonia which was slow in resolving. This led to investigation which revealed that he could not cough up his secretions because of weakness of half of his diaphragm. He too had had a severe reaction to tetanus antitoxin three years previously. A fourth patient was found by inquiry among our confrères.

These four patients who had paralysis of the diaphragm have in common only the history of preceding tetanus antitoxin serum neuritis. We believe that these cases merit reporting.

CASE REPORTS

CASE 1. A seventy-one year old white man was seen because of dyspnea on recumbency and pains in his arms. He stated that on July 1, 1951, he had cut his right heel badly enough to require surgical repair and he was given an injection of tetanus antitoxin. Five days later generalized urticaria developed which lasted only forty-eight hours, responding to antihistamine therapy. Following this severe pain developed in both arms, which still persisted at the time of his examination by us on August 22, 1951, some six weeks later. He had noted that the pain was worse in his right arm and shoulder, and that the grip of his right hand was weak. Dyspnea when recumbent, especially after a full meal or when stooping or bending, was noted at about the same time. He had had hypertension for five years, usually averaging around 160 to 170 mm. Hg systolic but occasionally rising to 200 mm. He had not had paroxysmal nocturnal dyspnea.

Examination revealed a well developed, elderly white man who was not cyanotic or dyspneic when erect but quite dyspneic in recumbency. The heart was slightly enlarged to the left, with slight accentuation of the aortic second sound and a faint systolic aortic murmur. The rate was 76 per minute, with regular rhythm. The lungs were clear. The abdomen was normal. The upper extremities revealed no clubbing or limitation of motion. There was slight weakness of the grip in his right hand. There was slight contraction of the palmar fascia in both hands. He had moderate benign prostatic hypertrophy. Fluoroscopic examination showed slight left ventricular hypertrophy and prominence of the aortic knob. The lung fields were clear. The right leaf of the diaphragm was elevated about three interspaces above the left and there were paradoxic excursions, due to phrenic nerve paralysis. A chest x-ray revealed some slight clouding of the right superior sulcus and the elevated right diaphragm. An electrocardiogram was within normal limits. Laboratory studies were not remarkable.

It was thought that he had a superior sulcus tumor with pressure on the right phrenic nerve and so he received x-ray therapy. He was given 1,700 r over the right upper anterior chest between August 28 and September 12, 1951. This produced no change in the patient's symptoms.

A chest x-ray made in January, 1949, was located. This showed the diaphragms were then in normal position. The vague density in the right superior gutter was visible and apparently was vascular in origin.

We continued to think of the possibility of serum neuritis producing phrenic paralysis. When the article by Comroe et al.[1] appeared, the diagnosis was changed in our patient. He has gotten along well since then. When examined on July 3, 1954, he had very few complaints except for prostatism. He has very little dyspnea now, even on recumbency, although fluoroscopy and chest films made on inspiration and expiration (Fig. 1) showed that paralysis of the right leaf of the diaphragm persists. His blood pressure was 210/100.

CASE II. A thirty-seven year old white man was first seen on July 15, 1952, because of pain in the right flank and a story of having passed bloody urine six weeks previously. This was found to be due to a calculus in the right ureter which was removed at cystoscopy by a urologist.

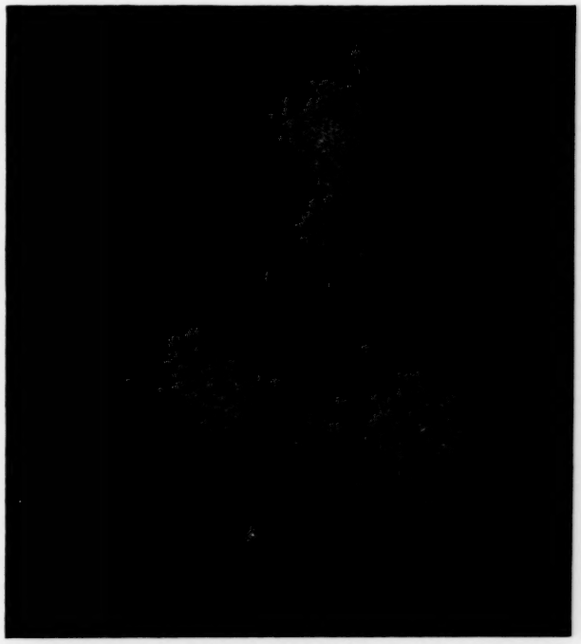
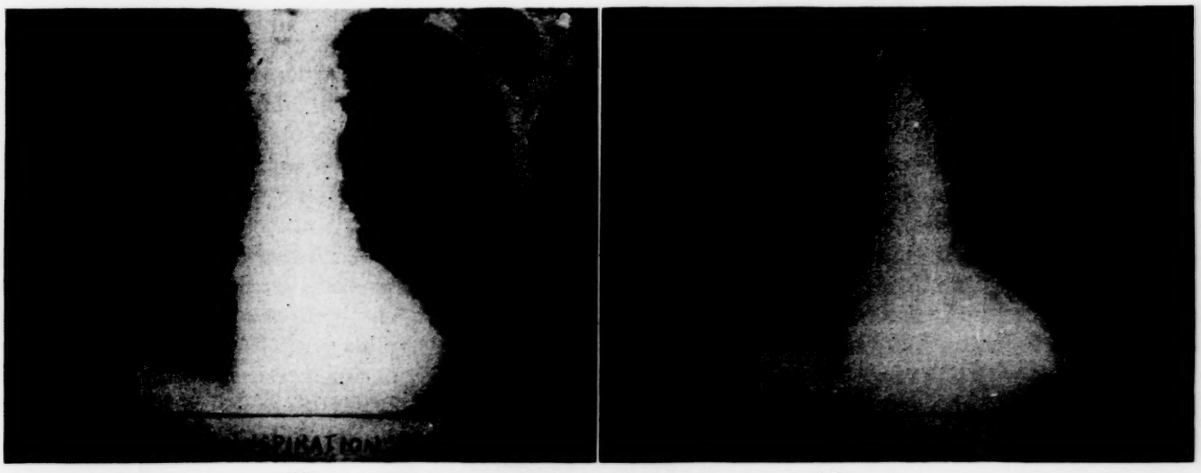


FIG. 1A. Patient No. 1 (August 22, 1951) showing paralysis of the right half of the diaphragm.



1B 1C

FIG. 1. B, July 3, 1954, deep inspiration. C, July 3, 1954, sniffing. The right half of the diaphragm ascends while the left side descends.

He also gave the story that in April, 1951, his truck had overturned. He was bruised but not otherwise injured. He was given an injection of tetanus antitoxin. Within a few hours he had urticaria around the waist and over the rest of his body. Four or five days later he had severe pains through the shoulders and was quite frightened. He felt as if he were becoming paralyzed. He remained home for a few days and then returned to work. He did not recall any definite weakness in the arms or shoulders following the accident or injection of tetanus antitoxin.

Physical examination revealed a well developed, healthy looking young man with no physical abnormalities except for partial paralysis of the left hemidiaphragm noted fluoroscopically and on the chest film. (Fig. 2.) The patient has been seen frequently since 1952 and was last seen January 16, 1954, at which time he was still in good health. The left diaphragm was still almost completely paralyzed while the right diaphragm moved well. There seemed to be a little compensatory emphysema of the right lung.

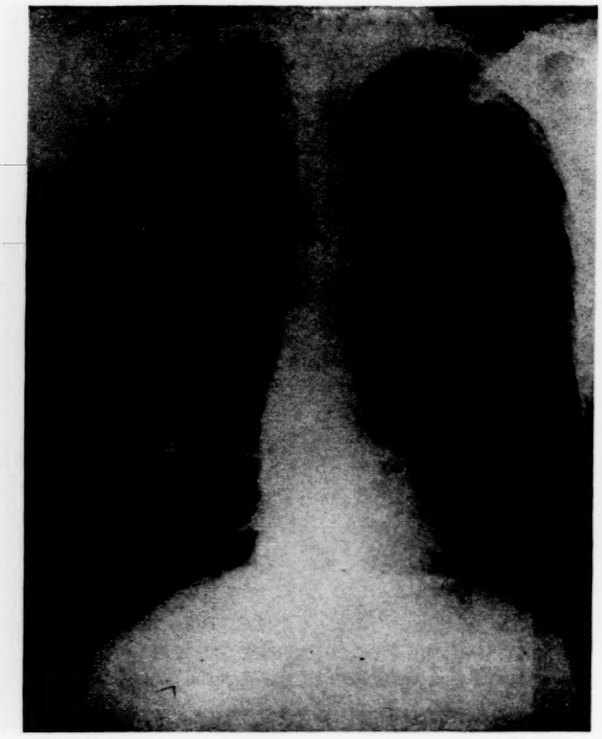


FIG. 2. Patient No. 2 (August 15, 1952). The left half of the diaphragm is paralyzed.

CASE III. A sixty-three year old white fireman was admitted to the hospital in coma on August 29, 1953. He had been in good health until ten days previously when he developed a cold which gradually became worse. The day of hospitalization fever and a chill developed and he soon lapsed into unconsciousness. Examination revealed an obese, comatose, critically ill man who was breathing with Cheyne-Stokes respirations. He was cyanotic. His pupils were pinpoint in size and did not react to light. The neck was supple. All peripheral tendon reflexes were absent. The lungs were full of rales and rhonchi. The heart sounds were almost inaudible, but the rhythm was regular and there were no murmurs or friction rubs. His blood pressure was 95/60. The abdomen was very obese with no palpable abnormalities. There was an old cholecystectomy scar. The optic fundi were normal after dilating the pupils. Lumbar puncture revealed clear fluid with normal pressure; it was reported to contain five cells, all lymphocytes, and 55 mg. per cent protein. X-ray of the chest revealed a small patch of pneumonitis at the right base. Urinalysis was normal. His red blood cell count was 4,660,000 per cu. ml. Hemoglobin was 14.8 gm. per cent. White cell count was 8,450

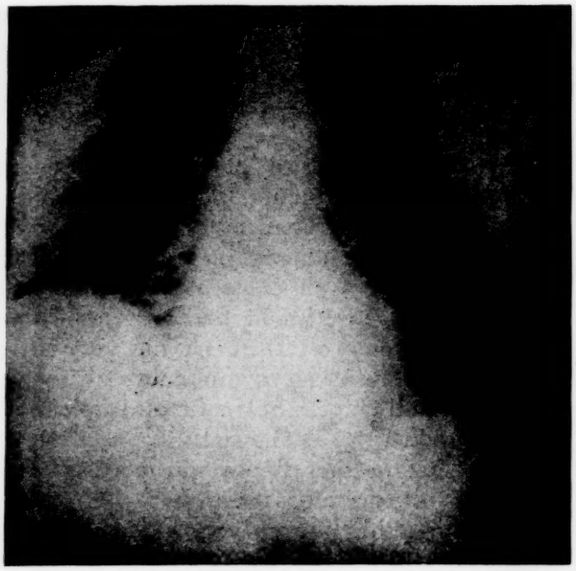


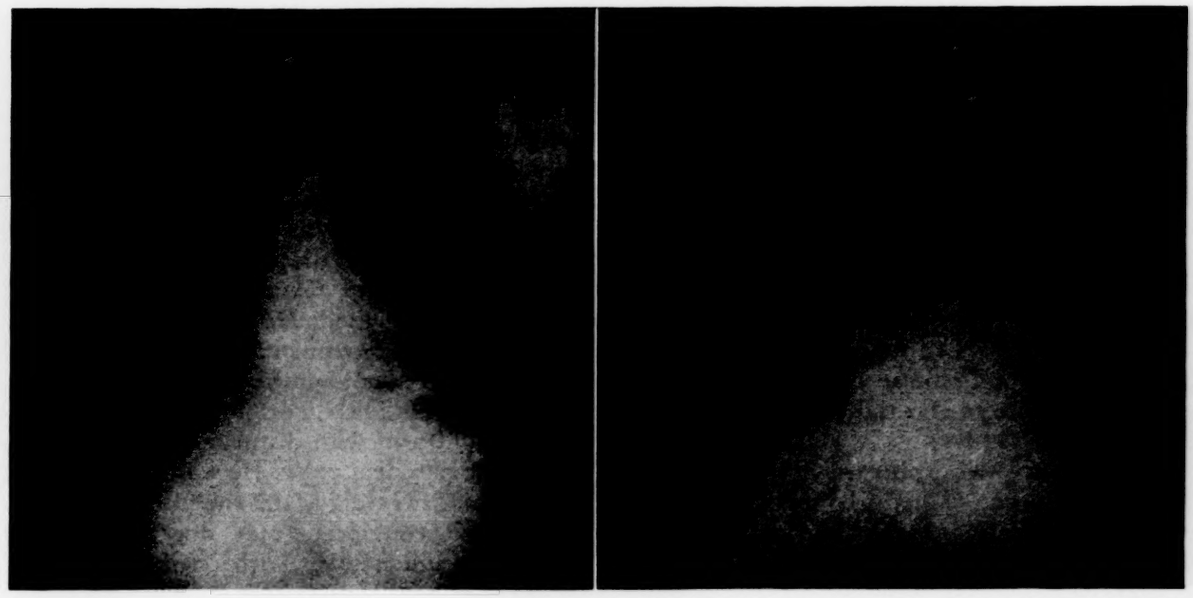
FIG. 3. Patient No. 3 (October 1, 1953). Paralysis of the right leaf of the diaphragm.

with 79 per cent segmented polymorphonuclear leukocytes, 5 per cent stab forms, one juvenile form, 2 per cent eosinophils and 13 per cent lymphocytes. His blood sugar was 158 mg. per cent, and the non-protein nitrogen was 83 mg. per cent. The carbon dioxide combining power was 48 volumes per cent.

The head of his bed was raised quite high and nasal oxygen was started. Within a few minutes he showed striking improvement with clearing of his sensorium and disappearance of the cyanosis. He was then treated with terramycin and adrenal cortical extract. He continued to improve rapidly and looked quite well the next day except for persistent cough. He had many rales in the right chest. His blood pressure was 138/70. He recovered smoothly and was discharged from the hospital in seven days.

Our attention was called to the diaphragm by the first chest film which revealed that the right diaphragm was several inches higher than normally expected. Further questioning revealed that he had had a reaction to tetanus antitoxin three years previously, with arthralgia and urticaria severe enough to require hospitalization. The patient remembered having a good bit of pain in the right hypochondrium and substernal area at the time but he did not remember any peripheral neuritis and we could find no written record of the incident. He had never had a previous chest x-ray.

At our request he came to our office October 1, 1953. He was getting along quite well. Fluoros-



4A 4B

FIG. 4. A, patient No. 4 (May 30, 1949), paralysis of the left leaf of the diaphragm. B, same patient (May 19, 1954). The left diaphragm descends a little more than in 1949.

copy revealed clear lung fields. Both diaphragms descended well on slow, steady inspiration, but on sniffing there was a clear-cut paradoxic motion with the right diaphragm ascending sharply while the left one descended. Films were made in inspiration and expiration. (Fig. 3.) On inspiration the left diaphragm descended 46 mm. while the right side descended only 6 mm. We believed that the evidence indicated definite weakness of the right side of the diaphragm and that it was probably due to injury to the right phrenic nerve as a result of his tetanus antitoxin serum neuritis.

CASE IV. A thirty-five year old white man had an injury to his left hand for which he received tetanus antitoxin on March 30 or 31, 1949. Three days later he noted swelling of the deltoid area of the left arm with redness and tenderness. On April 5th he awakened at 2 A.M. with severe pains in the arms. This soon spread over his shoulders and into the muscles of the lower part of his neck. It later spread to the upper part of his chest bilaterally. He lost the use of his arms to a considerable extent. The pain was intractable and not controlled readily by analgesic drugs. He gradually improved. Physical examination on May 30, 1949, revealed a little external strabismus of the left eye, diminished knee jerks, hypesthesia in both upper extremities, flexion contractures of the right hand, plus considerable limitation of usage of his arms and atrophy of the shoulder muscles, particularly of the right side. The accessory muscles of respiration were thought to be unaffected.

His family doctor remembers quite vividly that extreme difficulty in breathing was the patient's main complaint at the onset of his illness. Fortunately, a chest x-ray was made May 30, 1949, and this film revealed that the left diaphragm was higher than the right, contrary to the usual position. (Fig. 4.) This finding is compatible with weakness of the left side of the diaphragm due to phrenic nerve paresis.

Apparently most of the patient's neurologic defects have improved considerably since then. Physical examination on May 19, 1954, revealed that he still had some weakness and pain in his arms and shoulders. He had a minor reduction in sensation to cotton and pin prick in the right arm. The knee jerks were absent on both sides even with reinforcement. Fluoroscopically the leaf of the left diaphragm was now a little lower on inspiration than the right side. A film showed some weakness of the left side of the diaphragm, although there had been some return of function.

DISCUSSION

There is no way to prove beyond question that the paralysis of half of the diaphragm in each of these patients was due to tetanus anti-

toxin. However, we believe that the circumstantial evidence is strong.

We have listed four cases of persons in various age groups and with widely differing stories. The only points of similarity are the histories of receiving tetanus antitoxin followed by serum neuritis, and the later finding of diaphragmatic paralysis. In addition, the first three had typical urticaria and serum sickness preceding the serum neuritis. Only one of these patients had any clinically detectable residual paralysis of any peripheral nerves. The fourth patient still has some weakness and pain in his arms and shoulders, hypesthesia in the right arm and absent knee jerks.

Involvement of the phrenic nerve might be expected to occur rather frequently in serum neuritis complicating tetanus antitoxin therapy. For some reason this illness affects the fifth and sixth cervical segments preponderantly, usually producing the Erb-Duchenne type of paralysis. The phrenic nerve originates primarily from the fourth cervical segment, but receives a portion of its innervation from the third and fifth segments as well, so it could readily be involved. However, phrenic paralysis due to serum neuritis is not described in the standard textbooks of medicine or neurology, nor is it mentioned in the better summaries of the entire problem.[2–5] We believe that it has frequently been overlooked in the past when routine fluoroscopic examinations of the chest were uncommon.

Respiratory difficulty complicating serum neuritis has been reported infrequently. Dyspnea was listed as a complication of tetanus antiserum used in a twenty-three year old man by Lhermitte.[6] The only specific reference of this nature which we could find in the American literature was reported rather recently by Comroe et al.[1] Their patient had paralysis of the accessory muscles of respiration and weakness of both halves of his diaphragm as visualized fluoroscopically. He began to recover at six months and recovery was practically complete in two years.

Apparently the first report of phrenic nerve paralysis as a complication of serum neuritis was published in the German literature in 1927.[7] A very interesting case was reported by Ladstatter[8] in 1952. He reported a thirty-eight year old healthy man in whom tetanus developed as a result of an injury. Following the onset of lockjaw he was treated with large doses of tetanus antitoxin serum plus penicillin and avertin.® He was critically ill. On the eleventh hospital day serum sickness developed. Two days later his lockjaw disappeared and he began to improve. Then right lower lobar pneumonia developed, which cleared. Nineteen days after admission bilateral peroneal palsy was noted. Twenty-seven days after admission a chest x-ray revealed right phrenic paralysis with elevation of the right half of the diaphragm. Another film thirteen days later revealed that the phrenic paralysis had cleared. He was discharged from the hospital approximately three months after his illness began. When last seen, seven months after the onset of his illness, he still had weakness of the right arm with disturbances in sensation and some peroneal palsy. He was given a 50 per cent disability rating at this time.

We are convinced that our patients had tetanus serum neuritis with resulting paralysis of the diaphragm. Our first patient had a chest x-ray made two years prior to his tetanus reaction. The diaphragm appeared normal. When examined six weeks after the onset of the serum neuritis, the paralysis of his diaphragm was present and has remained unchanged ever since.

The second patient has had a persistent paralysis of the diaphragm for two years. He is in excellent general health otherwise. There is no other explanation except as a complication of his serum neuritis.

The third patient had pneumonia. He nearly died because his obese abdomen displaced his weakened diaphragm so high that it led to anoxia, particularly when his ventilation was compromised by the pneumonic process. When he was propped upright in the hospital bed, he promptly awakened as the weight of his stomach fell down from the diaphragm and permitted adequate air exchange. There was no other cause for the paresis of his diaphragm except the story of serum sickness and probable serum neuritis.

The fourth patient was found to have paralysis of the left leaf of his diaphragm fifty-five days after the onset of serum neuritis. His dyspnea appeared simultaneously with the serum neuritis. The paralysis of his diaphragm has improved but is still demonstrable in recent chest films.

Paralysis of the diaphragm is uncommon in otherwise healthy people; and when it is noted fluoroscopically, one is put on the alert for more serious illnesses, primarily neoplasms or injuries along the course of the phrenic nerve. A history

of onset at the time of tetanus serum neuritis could prove helpful in making one feel more complacent as to the cause of the paralysis and as to the prognosis. In our series of four patients, two have no apparent improvement at the end of two and three years, while the other two have shown partial recovery at the end of three and five years. Thus it appears that healing may return after a very long time and we should not be too pessimistic about the long-range outlook of these patients. Fortunately, most of them adjust to the paralysis and have no dyspnea on routine activity long before the nerve function has returned visibly.

SUMMARY

Four cases of paralysis of half of the diaphragm as a complication of tetanus antitoxin serum neuritis are reported. They are all in adult males.

Two patients have shown considerable improvement over a period of years, while the other two have failed to demonstrate any improvement at the end of two and three years.

Routine fluoroscopy of the chest should reveal more of these cases if the possibility of paralysis of the diaphragm due to this cause is kept in mind.

Acknowledgment: Case IV was reported through the courtesy of Drs. George Wilkinson and S. D. Campbell.

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Herpetic Meningo-encephalitis Accompanying Cutaneous Herpes Simplex*

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MENINGO-ENCEPHALITIS is perhaps the least common of the variety of clinical manifestations resulting from infection with herpes simplex virus.[1] In some instances of this disease involvement of the nervous system only is evident. In others a preceding or accompanying herpetic infection of the skin or mucous membranes constitutes the source from which invasion of the central nervous system proceeds. The general impression that it is nearly always fatal is erroneous. Recent serologic studies[2,3] indicate that an increase in antibody to herpes simplex virus can be demonstrated during the course of some apparently benign cases of "aseptic meningitis." Convincing confirmation for the causative role of herpes simplex by isolation of the virus from the spinal fluid combined with serologic evidence has been achieved in only a few cases.

This report will present the clinical and laboratory observations which were made in a patient with herpetic infection in whom meningo-encephalitis was a prominent feature. The study was continued during convalescence and at various intervals for a year after onset of the disease. A considerable amount of interesting and instructive information was obtained regarding the behavior of the virus and the immune response of the patient during and following the episode in which the central nervous system was invaded. The experience which was gained may prove to be helpful in anticipating the problems and difficulties of such study when other cases of this type are encountered.

CASE REPORT

Present Illness. W. G., a thirty-six year old white newspaper reporter, was admitted to the Veterans Administration Hospital in New Orleans, Louisiana, on September 26, 1951. His immediate difficulty had begun four days previously as a sharp constant pain in the lower lumbar region radiating over both buttocks, the posterior aspects of the thighs and the calves, to his heels. This persisted for two days. On the day following the onset of "sciatica" he began to have fever from 99° to 102°F., frequent chilly sensations without frank chills, severe frontal headache spreading over the vertex, soreness of the neck, aching and tenderness of the eyes, and photophobia. He lost his appetite and experienced frequent bouts of nausea. In the evening a fever of 102°F. accompanied by drenching sweat occurred, after which the headache and fever moderated for a short time. He never became irrational or lethargic but complained of difficulty in concentrating his thoughts because of the severe headache.

This episode had been preceded by the development of two peculiar skin lesions which had begun five weeks previously. The first was noted as a small abrasion of the penile foreskin. Another papular lesion, which developed two days later on the left side of the face, was attributed to a mosquito bite. Within a week both lesions had become vesicular and purulent. Treatment with zinc oxide and 5 per cent sulfathiazole ointment did not interrupt their progression and considerable extension. Two weeks after the onset of the skin lesions a physician was consulted. Scrapings from the penile lesion revealed neither spirochetes nor Hemophilus ducreyi. A serologic test for syphilis was negative. After observation for ten days he was given 2 gm. of terramycin daily for four days without appreciable benefit. The symptoms which were immediately responsible for his hospitalization began three days after cessation of the terramycin therapy.

Past History. The outstanding feature in the patient's past history was the discovery of several

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suspicious areas of increased density in both lung fields in the routine chest film at the time of his discharge from the Navy in 1945. He was therefore hospitalized for a two-month period, during which careful clinical and laboratory studies yielded no definite diagnosis. The tuberculin test was negative. He was considered as probably having Boeck's sarcoid. Periodic chest films subsequently revealed increasing fibrotic infiltration bilaterally, most marked in the subapical regions. In 1949 he was hospitalized here because of a spontaneous pneumothorax on the right. At this time the chest x-ray, moderate elevation of serum globulin and a negative tuberculin reaction again suggested the diagnosis of Boeck's sarcoid. Since no superficial lymph nodes were enlarged, a biopsy to substantiate the diagnosis was not done.

A special point was made to ascertain whether or not the patient had had "fever blisters" or other lesions compatible with herpetic infection before the onset of the present illness. No such history could be obtained.

Physical Examination. The patient seemed to be acutely ill. He complained of severe headache. The temperature was 100.2°F., pulse 100, respiration 22 and blood pressure 118/70. The neck was not stiff. The pharynx was diffusely red. Moderate tenderness could be elicited on palpation of the left upper abdominal quadrant. On the left lateral aspect of the foreskin a red, edematous area measuring 3 by 1 cm. was present, the surface of which consisted of multiple small shallow ulcers. No vesicles, exudates or crusts were present at this time. Slightly above and anterior to the angle of the left mandible there was a 2 by 2 cm. area of skin which was crusted over, slightly purulent and apparently secondarily infected. Immediately above this area two small vesicles containing clear fluid were noted.

Neurologic examination revealed absence of the left ankle jerk. The right ankle jerk was hyperactive, with poorly sustained clonus. Babinski's sign was absent. No sensory abnormalities were noted. The retinas appeared to be normal.

Laboratory Studies. The initial lumbar puncture revealed a pressure of 112 mm. H_2O, and yielded clear spinal fluid containing 15 neutrophils and 195 lymphocytes per cu. mm. and 110 mg. per cent of protein. Examination of the blood showed 5.2 million red cells and 16,150 white cells per cu. mm., with 80 per cent polymorphonuclears, 18 per cent lymphocytes and 2 per cent eosinophils. Urinalysis revealed no abnormalities.

No bacteria or fungi were recovered from blood and spinal fluid cultures obtained on admission. Febrile agglutinations provided no significant information. Serologic tests for syphilis were negative. Initial cultures from the facial lesions yielded Staphylococcus aureus hemolyticus only. Neither H. ducreyi nor spirochetes could be demonstrated in scrapings from the penile lesion.

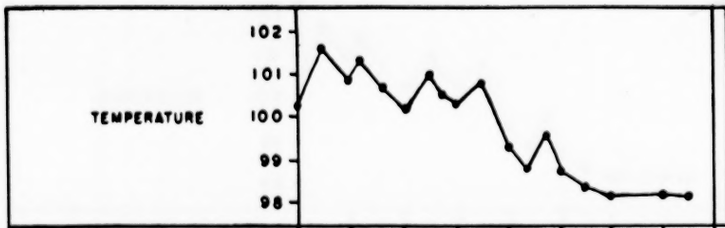
Isolation of Herpes Simplex Virus during Hospitalization. The recovery of herpes simplex virus from the spinal fluid obtained on September 28th indicated the specific nature of the patient's neurologic symptoms and gave a clue to the possible etiology of the skin lesions. Isolation and identification of the virus was accomplished by October 16th. Herpes simplex virus was subsequently recovered from scrapings obtained from the facial lesion on October 19th and from the genital lesion on October 25th. No virus could be isolated from the spinal fluid or scrapings from the facial lesion obtained on October 25th. The spinal fluid findings, white blood count determinations and virus isolations are presented in Table 1.

Course in the Hospital. During the first four days of hospitalization the patient's temperature fluctuated between 100.2° and 101.6°F. The headache, photophobia, anorexia and nausea gradually subsided. During the next three days the temperature steadily decreased to normal. From October 1st until November 25th when he was discharged there was no recurrence of fever or of the other symptoms mentioned.

The facial and genital lesions persisted without evident change throughout the first three weeks of hospitalization except that the vesicles accompanying the facial lesion disappeared during the first week. From September 28th to October 6th he was treated with 0.5 gm. of terramycin every six hours after an initial dose of 1 gm. From then until October 20th 300,000 units of aqueous procaine penicillin twice daily replaced the terramycin. This was then discontinued and aureomycin-varidase ointment was applied locally. From this time on the lesions gradually disappeared. The foreskin healed completely by November 1st and the face was clear by November 25th.

The absence of the left ankle reflex persisted throughout the patient's hospital stay. The

TABLE 1



DAY OF HOSPITALIZATION		1	2	3	4	5	6	7	8	16	22	24	28	30	37	44	51	62	76*	107*	118*	276*
DATE		9/26/52	9/27	9/28	9/29	9/30	10/1	10/2	10/3	10/11	10/17	10/19	10/23	10/25	11/1	11/8	11/15	11/26	12/10	1/10/52	1/21/52	6/30/52
SPINAL FLUID	PRESSURE (mm. H_2O)		112	320					Normal	N.D.	N.D.			Normal	200	190	160	150	170		130	130
	NEUTROPHILS per cu. mm.		15	30					5	0	0			0	0	0	0	0	0		0	0
	LYMPHOCYTES per cu mm.		195	180					215	200	90			50	40	40	25	5	5		10	0
	PROTEIN Mgm. %		110	96					128	146	179			142	107	81	74	68	65		66	56
BLOOD	W B C	16,150	11,750				10,800						10,500		7900							
	% NEUTROPHILS	80	79				79						92		83							
	% LYMPHOCYTES	18	18				12						8		15							
ATTEMPTS TO ISOLATE HERPES SIMPLEX VIRUS	CSF			+										0					0		0	0
	FACIAL LESION											+		0								
	GENITAL LESION													+								
	LESION ON LEFT HEEL																			+		

Explanation for symbols: *represents outpatient visits; N.D., not done; +, herpes simplex virus isolated; 0, no herpes simplex virus isolated.

ankle clonus on the right was not noted again after the first day in the hospital. On October 19th he had double vertical vision for a few minutes and for the next four days noted blurring of vision on rapid turning of the head to the right. This did not occur when he turned his head slowly. Examination of the fundi, determination of the visual fields, and tests of extraocular muscle functions at this time revealed no abnormalities.

During this period of hospitalization further studies were made with reference to Boeck's sarcoid. An x-ray revealed very little change in the areas of fibrosis scattered through both lung fields. A needle biopsy of the liver was performed. Microscopically, granulomatous lesions compatible with those of Boeck's sarcoid were found. Liver function tests were within normal limits except bromsulphalein retention, which initially was found to be 3 per cent but in mid-November was recorded as 12 per cent in forty-five minutes. Repeated serum albumin and globulin determinations were normal. The tuberculin test was again negative.

Subsequent Course. The patient has been seen at irregular intervals after his discharge in order to ascertain the state of his sarcoidosis and to follow the manifestations of the herpetic infection. He returned on January 10, 1952, with a crop of vesicular lesions over the left heel from which herpes simplex virus was isolated. These lesions persisted for approximately two weeks. Since then he has continued to have recurrences of vesicular eruptions every two to three months. Except for one lesion of the right cheek, these have occurred only in the area of the old lesion on the left cheek, and at scattered sites over the lower extremities, most often the left lower extremity. The lesions on the legs usually remain vesicular for a few days and then become crusted over, lasting about three weeks altogether. In contrast, those on the face generally heal completely within four or five days. The recurrent lesions have been assumed to be herpetic on the basis of their appearance. No attempt at virus isolation has been made since January 10, 1952.

In May, 1952, he began to have a rather ill defined aching of the left shoulder and arm occurring every four to six hours. Although readily relieved by aspirin, this indisposition has continued until the present time. Repeated x-rays of the bones have been negative and no cause for the pain has been found.

The left Achilles tendon reflex has continued to be absent. No other neuromuscular abnormalities have developed. Spinal fluid cell counts and protein determinations made since discharge from the hospital have remained within normal limits. Repeated laboratory observations, with particular reference to the sarcoidosis, have shown a slowly rising blood urea nitrogen (present level 29 mg. per cent) and bromsulphalein retention (17 per cent in forty-five minutes). The electrophoretic pattern of a recent serum sample was normal.

VIRUS ISOLATION AND SEROLOGIC STUDIES

Isolation of Herpes Simplex Virus

Spinal Fluid, Sept. 28, 1951. Because of the presence of the genital lesion, lymphogranuloma venereum virus was at first strongly considered as a possible etiologic agent. The initial attempts at virus isolation were directed to this purpose. Spinal fluid in 0.03 cc. amounts was injected intracerebrally into each of four C.F.W. strain mice and in 0.5 cc. amounts into the yolk sac of each of six eight-day old chick embryos.

Of the five embryos surviving longer than twenty-four hours, two died on the sixth day. The presence on the seventh day of pock-like lesions on the chorio-allantois of one of the remaining three was the first indication that herpes simplex virus should be considered. Subsequent microscopic study of this membrane revealed Type A intranuclear inclusions in ectodermal epithelial cells in the focal lesions. A strain of herpes simplex virus was established in the chorio-allantois of eleven- to fourteen-day old embryos. The yolk from one of the embryos dead on the sixth day served as the initial inoculum.

One of the four mice exhibited signs of encephalitis on the tenth day. The other three remained normal and six weeks later were shown to be susceptible to challenge inoculation with the strain of virus derived from the patient's spinal fluid. From the brain of the mouse with encephalitis a strain of herpes virus was isolated and maintained through nine successive intracerebral passages in mice, after which the series was intentionally terminated. Most of the mice in each passage died on the fourth or fifth day after inoculation.

Spinal Fluid Obtained Oct. 25, 1951, December 10, 1951, Jan. 21, 1952, and June 30, 1952. Herpes simplex virus could not be recovered from the

first three samples of this series by inoculation of the chorio-allantois of twelve-day embryos. Three to five blind passages were made before considering each test as negative. The June 30th specimen did not produce signs of encephalitis in six mice during three weeks of observation.

Facial Lesion, October 19, 1951. Swabbings from the base of the lesion suspended in 1 cc. saline containing penicillin and streptomycin produced typical herpetic lesions in the chorio-allantois of twelve-day chick embryos within seventy-two hours after inoculation. Microscopic sections from one of the membranal lesions revealed Type A intranuclear inclusions. A strain of virus was established from this source by passage in the chorio-allantois of eleven- to thirteen-day old embryos.

Facial Lesion, Oct. 25, 1951. Herpes simplex virus was not recovered by inoculation of the chorio-allantois from swabbings collected on this date.

Genital Lesion, Oct. 25, 1951. Vesicle fluid absorbed on a sterile cotton swab and suspended in 1 cc. saline containing penicillin and streptomycin was inoculated on the chorio-allantois of twelve-day embryos. Characteristic membranal lesions appeared in the second passage from this source. Typical intranuclear inclusions were found in sections of the membranal lesions.

Heel, Jan. 10, 1952. Vesicle fluid treated in the same manner as that collected from the genital lesion produced characteristic herpetic lesions on the first passage in the chorio-allantois of twelve-day embryos. Type A intranuclear inclusions were found in sections through the membranal lesions.

Identification of the Virus as Herpes Simplex

A strain of virus derived from each of the four sources in the patient was maintained by passage in the chorio-allantois. Virus neutralization was done by the "pock counting" method, using antiserum prepared by hyperimmunization of chickens with H.F. strain of herpes virus. Previous tests had established a final 1–20 dilution as its effective neutralization titer against several strains of virus obtained from different individuals with herpetic infections. The supernatant fluid obtained by centrifuging a 10 to 20 per cent saline suspension of heavily infected membranes for ten minutes was mixed in equal amounts with a 1–10 dilution of the test serum and incubated at 35°c. for two hours. Virus suspension in an equal amount of sterile saline constituted the control. Each of seven or eight twelve- to fourteen-day embryos was inoculated with 0.2 ml. of the mixtures, evenly spread over the chorio-allantois by means of a tuberculin syringe and needle. Pock counts were made at forty-eight to seventy-two hours following inoculation. The results are presented in Table II.

It is apparent that this method produces considerable variations from one test run to another and also varies markedly from embryo to embryo in each test. Taken as a whole, the neutralization tests offer fairly good evidence for identifying the four strains of virus as herpes simplex. The absence of more complete neutralization can be accounted for by a more recently completed study, employing complement fixation, which indicates that this strain is antigenically quite distinct from the H.F. and other strains obtained from various sources.[4]

Behavior of the Virus in Rabbits. The corneas of each of two rabbits were inoculated with a membranal suspension from an early passage of the virus derived from the spinal fluid. Characteristic herpetic keratitis and conjunctivitis developed in each animal. One was sacrificed at forty-eight hours. Typical intranuclear inclusions were demonstrated in the corneal epithelium. The other animal exhibited signs of encephalitis on the fourth day and died on the seventh day following inoculation. Typical herpetic encephalitis was also produced in two rabbits following corneal inoculation with membrane passage virus derived from the heel lesion.

Studies of the Patient's Antibody Response to Herpes Simplex Virus

Fourteen serum samples were obtained at intervals of varying duration between October 5, 1951, and November, 1952. They were stored in sterile rubber-stoppered tubes at 5°c. until used. Virus neutralization tests were performed with nine of the samples of serum collected between October 5, 1951, and October 27, 1952. Complement fixation tests were run on thirteen samples obtained between October 15, 1951, and November 24, 1952. The strain derived from the patient's spinal fluid was used as test virus in these determinations.

Neutralization Tests on the Patient's Serum. Virus suspensions: The yolk sac of eight-day embryos was inoculated with 0.2 ml. of the supernatant fluid obtained by centrifuging a 10 to 20 per cent suspension of infected membranes from the

fourteenth passage. The amniotic fluid collected from embryos dead on the fourth or fifth day was cultured, pooled after forty-eight hours when found to be free of bacterial contaminants and centrifuged at 2,000 r.p.m. to remove coarse particles. Normal inactivated rabbit serum was added to a final concentration of 10 per cent. This preparation was distributed in equal amounts to glass ampules, flame-sealed, quick frozen and stored at −20°C. until used. Another lot was prepared in the same manner from the fifteenth membranal passage. These are referred to in Table III as Lots A and B, respectively. Each lot was used for about one month after freezing.

Technic of the Test. Phosphate-buffered saline, pH 7.4, containing 10 per cent normal inactivated rabbit serum, constituted the diluent for the virus suspension and the serum in all tests. The test sera, diluted 1:10, were heated at 56°C. for thirty minutes on the day of the test. Preliminary titrations indicated that 0.2 cc. of a final 1–400 dilution of Lot A and the same amount of a final 1–1,000 dilution of Lot B could be expected to produce an average of 100 to 200 pocks on the chorio-allantois of twelve-day embryos. Equal amounts of a 1–200 dilution of Lot A and a 1–500 dilution of lot B and a selected range of fourfold dilutions of the serum samples were mixed and incubated at 35°C. for two hours. A normal serum-virus control mixture was prepared in the same manner with a 1–10 dilution of human serum previously demonstrated to contain no neutralizing antibodies to herpes simplex virus. Each of six twelve-day embryos was inoculated with 0.2 cc. of each serum-virus mixture, spread evenly over the chorio-allantois by means of a tuberculin syringe and needle. Pock counts were made after forty-eight hours' incubation at 35° to 37°C. The results are presented in Table III.

TABLE II

IDENTIFICATION OF THE PATIENT'S VIRUS STRAINS BY NEUTRALIZATION TESTS

Date of test	10/24/51		1/25/52		12/22/51		1/22/52	
Source of virus	Spinal Fluid		Facial Lesion		Penile Lesion		Heel Lesion	
No. of embryo passages prior to neutralization test	Mouse 2 → Embryo 1		4		5		3	
Age of embryos	14-Day		13-Day		14-Day		12-Day	
Approximate pock counts	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum	Saline Control	H.F. Antiserum
Embryo No. 1	>200	3	>300	>200	>200	30	>300	>200
No. 2	>300	20	>300	150	>200	15	>300	>300
No. 3	>300	4	>300	200	>200	50	>300	50
No. 4	0	1	>300	100	>200	10	>300	100
No. 5	60	100	>200	200	>200	>200	200	75
No. 6	>300	25	>200	100	>200	>200	>300	250
No. 7	>300	15	>300	>300	>200	25	>300	75
No. 8	Dead	25	.....	.....	>200	>200	.....	.....

It is to be emphasized that the "pock counting" technic provides information only as to relative differences in neutralizing antibody levels. It is not sufficiently exact to provide strict quantitative comparisons between different serum samples.

The results of these tests nevertheless indicate that herpes neutralizing antibody in low concentration was present in the first sample of serum collected during the time the patient had meningitis. The antibody concentration increased slowly during the following five weeks

and then dropped to low levels. It is interesting to note that the titer was found to be low on January 10, 1952, at the time of recurrence of the cutaneous herpes over the heel. The subsequent rise observed by January 21, 1952, apparently represented a response to the recurrence of the infection at that time. It is difficult to interpret the significance of the antibody levels obtained on June 30, 1952, and October 27, 1952, in relation to the frequent recurrences of cutaneous infection experienced by the patient.

TABLE III

TITRATION OF PATIENT'S SERUM SAMPLES FOR HERPES SIMPLEX VIRUS NEUTRALIZING CAPACITY BY THE "POCK COUNTING" TECHNIC

Serum date		10-5-51	10-15-51	10-25-51	11-15-51	12-10-51	1-10-52	1-21-52	6-30-52	10-27-52
Virus lot		A	A	A	A	B	A	A	B	B
Date of test		10-27-52	10-21-52	10-27-52	10-21-52	11-20-52	10-28-52	10-28-52	11-19-52	11-23-52
Final serum dilution	1:20	7/160	22/166	13/160	8/166	6/116	19/151	1/151	0/152	0/97
	1:80	59/160	51/166	31/160	19/166	32/116	37/151	5/151	1/152	1/97
	1:320	86/160	84/166	46/160	33/166	48/116	47/151	36/151	8/152	22/97
	1:1280	.....	.....	86/160	91/166	155/116	.....	72/151	46/152	100/97
	1:5120	.....	.....	.....	.....	.....	.....	.....	117/152	113/97
Serum titer*		1:39	1:34	1:92	1:320	1:49	1:45	1:246	1:735	1:265

Numerator—Average pock count obtained with the serum dilution.
Denominator—Average control pock count.
* Serum titer expressed as the dilution estimated by interpolation to produce 80 per cent reduction in pock count.

Complement Fixation Tests. Thirteen serum samples collected between October 15, 1951, and November 24, 1952, were tested for their capacity to fix complement with herpes simplex antigen. In general, the technic followed was that described by Enders and Levin,[5] employing 0.1 cc. each of antigen and serum and 0.3 cc. of complement. After fixation overnight in the refrigerator at 5°c. the tests were completed by addition of 0.25 cc. of sensitized 1 per cent sheep cells. After incubation for thirty minutes in the 37°c. water bath, tests were read immediately. The highest serum dilution giving 3+ (50 per cent) or greater fixation was then taken as the endpoint.

All dilutions were made in 0.85 per cent NaCl solution containing 100 mg. $MgSO_4$ per L. Pooled serum from four to six guinea pigs, stored in small aliquots at −20°c. and used within five days after collection, constituted the source of complement. A washed 2 per cent sheep cell suspension sensitized with an equal volume of 1:1000 dilution of commercial anti-sheep cell hemolysin was used as the indicator system for all complement titrations and tests. Complement was titrated on each day that tests were set up to determine the dilution which would give two hemolytic units in 0.3 cc. The actual dilution used in the tests varied from 1:53 to 1:100.

The strain of herpes simplex virus derived from the patient's spinal fluid served as the antigen. Considerable difficulty was encountered in obtaining a satisfactory stable antigen suspension with which comparable results could be obtained. Amniotic fluid aspirated from embryos dying three to four days after inoculation into the yolk sac seemed to be best suited to this purpose. Bacteria-free fluids were pooled, centrifuged at 1,500 r.p.m. for fifteen minutes, stored at 5°c. and used within two to three days. The antigen suspension was titrated in serial two-fold dilutions against a 1:4 dilution of each of three positive serum samples obtained from individuals with recurrent herpes. The highest dilution of antigen giving complete fixation with two of the three sera was designated as "1 unit" and twice this amount was then used for the tests. As noted in Table IV, only one antigen suspension was used in a dilution higher than 1:2.

The serum samples were all tested simultaneously, using the same reagents. There was thus considerable variation in the length of storage of different samples. Several samples were found to be anticomplementary. Before each test, aliquots of a 1:4 dilution of each serum were heated at 60°C. for twenty minutes and then reheated at 62°C. for fifteen minutes. Twofold dilutions ranging from 1:4 to 1:128 were set up, using a clean pipette for each dilution. A 0.1 cc. amount of each dilution, delivered to a clean tube, was used for the test.

TABLE IV

COMPLEMENT FIXATION TITERS OF SERUM SAMPLES COLLECTED FROM OCTOBER 15, 1951, TO NOVEMBER 24, 1952

Date of Test	Antigen Dilution	Date of Serum Sample												
		10-15-51	10-25	11-8	11-15	11-27	12-10	1-10-52	1-21	6-30	8-25	9-29	10-27	11-24
8-21-52	1:8	1:8	1:8	1:8	1:8	1:8	—	<1:4	—	1:64	—	—	—	—
1-9-53	1:2	1:32	1:32	AC	1:64	1:64	AC	1:16	AC	AC	1:64	AC	1:64	1:64
1-19-53	1:2	1:16	1:32	—	1:16	1:16	—	1:4	—	—	1:32	—	1:32	1:32
2-9-53	1:2	1:8	1:16	—	AC	AC	—	1:4	—	—	AC	—	AC	1:32

AC represents anticomplementary; —, not done.

The following controls were included in each test: (1) Serum controls: (*a*) serum plus normal amniotic fluid in 1:2 dilution; (*b*) serum alone; (*c*) serum plus 2 units of complement; (*d*) serum plus 1 unit of complement. Serum which produced more than 1+ fixation (10 per cent) with 1 unit of complement was considered anticomplementary. No fixation took place in the presence of normal amniotic fluid. The negative human serum control was from the same individual providing the negative control in the neutralization tests. (2) Antigen controls: (*a*) antigen only; (*b*) antigen plus 2 units of complement; (*c*) antigen plus 1 unit of complement. The normal amniotic fluid was set up with the same controls. None of the antigen suspensions or normal amniotic fluids was found to be anticomplementary in the dilutions used. (3) Complement controls: 1 unit and 2 unit complement controls were included to test for complement deterioration during the overnight period in the refrigerator at 5°C.

Table IV presents the results obtained by the complement fixation reaction.

The results of the complement fixation tests demonstrate the difficulties encountered in the application of this method to a series of serum samples collected at intervals over a long period of time. At ordinary refrigerator temperatures serum is likely to become anticomplementary upon prolonged storage. This interferes with attempts to measure the complement fixing titer of the entire group of samples against a single antigen preparation. Considerable variation in results is further encountered when the same serum samples are tested with different antigen preparations at successive intervals. A stable antigen preparation is not yet available which can be used to determine the titer of each serum sample within a short time after its collection. The results here presented permit only the limited conclusion that complement fixing antibodies could be demonstrated in successive serum samples drawn from the patient at intervals between October 15, 1951, and November 24, 1952. There appears to be some fluctuation in complement fixing titer during this period.

Serologic Tests with Other Viral Agents. Serum samples collected on October 5, October 10, October 25 and November 15, 1951, were sent to the Communicable Disease Center Laboratories in Montgomery, Alabama. No complement fixing antibodies for mumps, lymphogranuloma venereum, eastern and western equine encephalomyelitis or lymphocytic choriomeningitis could be demonstrated in these specimens.

COMMENTS

The specific nature of this patient's illness became evident when lesions compatible with those produced by herpes simplex virus were

observed in embryonated eggs inoculated with spinal fluid obtained during the early stages of the meningo-encephalitis. This observation led to isolation of the virus from the cutaneous lesions. Clinically, the involvement of the central nervous system appeared to be a self-limiting process. The cutaneous eruption can be considered to be the primary source for infection of the central nervous system. The serologic studies, which demonstrate a gradual but definite increase in specific antibody titer, provide further evidence that herpes simplex virus was the cause of the infectious process.

It seems reasonable to believe that this was a first or "primary" infection. No history could be established to indicate the occurrence of previous "fever blisters" or other manifestations of herpetic infection. Since the first serum specimen tested was obtained five weeks after onset of the initial cutaneous lesion, examination of an "acute" specimen was not possible. The demonstration of the absence of antibodies in the acute serum specimen would have provided more satisfactory evidence for the "primary" nature of the process.

Problems regarding the pathogenesis of the central nervous system infection and the recurrent skin eruptions provide interesting speculation in the light of the available data. At the time central nervous system involvement was evident, circulating antibodies could be demonstrated. If invasion by the blood stream accounted for this localization, it apparently took place early in the course of the initial cutaneous eruption. It is unlikely that a viremia would persist for any length of time when specific antibodies, presumably developing in response to the cutaneous infection, had made their appearance in the blood stream. Invasion by propagation along nerve axons from the initial, or genital, lesion would probably be less affected by circulating antibodies. Clinical evidence, particularly the sciatic nerve pain, gives support to the idea that a centripetal spread from the initial lesion along nerve tracts may have constituted an important phase in the pathogenesis of the central nervous system involvement. There is good reason to explain the skin eruption over the left heel as resulting from a centrifugal spread of the virus along nerve tracts. The regular recurrence of cutaneous lesions is perhaps best explained on the basis of persistence of the virus in the central nervous system.

The strain of herpes virus isolated from the spinal fluid of this patient has proved to be of considerable interest. Neutralization tests with anti-H.F. serum suggested divergence in antigenic characteristics. This observation has led to a detailed antigenic analysis by complement fixation of several strains of herpes virus obtained from patients with other manifestations of herpetic infection.[4] It has become evident that this strain is antigenically quite distinct from the majority of the others thus far compared. Whether or not this antigenic difference reflects a difference in behavior of the virus, as indicated by the long duration of the cutaneous lesions, is a matter for speculation. It is interesting to note that the recurrent lesions of the face follow a course more like that of the usual recurrent labial herpes than do those of the lower extremities, which are of a more protracted nature. The pathogenesis of these episodes in relation to the patient's immune response remains an unsolved problem. In what manner, if any, the sarcoidosis may alter this patient's reaction to herpetic infection is not evident. Answers to these questions await satisfactory elucidation of the pathogenesis of the more common types of recurrent herpes simplex.

The experience gained from this case emphasizes the importance of previous familiarity and precedent as a guide in the study of cases of this type. Long-term comparative serologic studies are especially difficult. Quantitative comparisons of the antibody titer by means of complement fixation tests are made difficult by the tendency of serum to become anticomplementary on storage at ordinary refrigerator temperatures. Storage of sera in the frozen state is probably more satisfactory from this standpoint. A stable antigen preparation has not yet been devised. Although neutralizing antibodies are more likely to remain stable on storage, strict quantitative comparisons of neutralizing antibody levels are difficult to achieve. The serologic data obtained from this case indicate that the antibody level against herpes simplex fluctuated considerably. The relation of these fluctuations to the recurrent episodes of cutaneous infection is not clear in every instance. The answer to this and other problems awaits more thorough and consistent study of other cases.

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Fibrinolytic Purpura in Acute Leukemia*

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In a number of different diseases the abnormal occurrence of active fibrinolytic enzymes in the blood may result in severe bleeding.[1–18] Enzymatic dissolution of blood clots with hemorrhage has been described in traumatic shock,[1,2] following thoracic[3,5,6] and abdominal[3,4] surgery, in pathologic pregnancy such as Rh incompatibility[7,8] or abruptio placenta,[9,10] in severe liver disease,[11] in septicemia[12] and as a complication of blood transfusion.[13,14]

The association of fibrinolysis with certain types of malignancy has been noted recently.[15–18] It has been demonstrated[16,19] that prostatic cells secrete a proteolytic enzyme, in effect identical with fibrinolysin, so that an increase in prostatic neoplastic tissue mass or its dissemination throughout the body might result in an increase in the number of functioning cells that produce this substance. Fibrinolysis has neen noted also in association with carcinoma of the pancreas[17,18] but a similar relationship between a fibrinolytic enzyme and the pancreatic secretion has not yet been demonstrated.[17]

Fibrinolysis in leukemia is little known, having been the subject of only a few reports. Janbon et al.[20] described a five year old girl who had leukemia associated with bleeding attributed to thrombocytopenia and to a total absence of fibrinogen. These authors did not report actual lysis of the clot by a fibrinolysin. In June 1954, Giraud and his group[21] reported the case of a forty-five year old man with acute granulocytic leukemia in whom hemorrhage was associated with thrombocytopenia as well as enzymatic dissolution of the fibrin clot. In a study of fifty cases of leukemia of various types, Mene et al.[22] demonstrated increased activity against fibrinogen *in vitro* in the plasma of forty-six patients. Zardi[23] demonstrated a similar fibrinogenolytic phenomenon in ten of eleven cases of Hodgkin's disease. In these experiments, sterile oxalated plasma was incubated for four days and daily determinations of fibrinogen were done. Although a progressive fall in the plasma fibrinogen occurred following incubation, none of these patients showed a bleeding tendency and there was no lysis of the fibrin clot once it was formed. Interference with fibrin formation by a peculiar protein was recently described in a case of multiple myeloma, but true fibrinolysis was not described and the patient apparently did not bleed.[24] No further report of fibrinolysis in leukemia has been made with the exception of passing reference to the possibility of this phenomenon in a review on fibrinolysis.[25]

In this report we wish to describe a patient with leukemia in whom a fibrinolysin was demonstrated shortly before death. Although a more complete study of the properties of the fibrinolytic enzyme could not be carried out, the occurrence of fibrinolysis in leukemia is of such clinical import as to call attention to its presence.

CASE REPORT

Patient J. S. (MCGH, 444412), an eighty-two year old man, was first seen in July 1949 at which time a small rectal polyp was removed. This proved to be an adenocarcinoma of low grade malignancy. No evidence of metastasis was present at the time of surgery. The blood count on this admission was within normal limits. Follow-up studies in the outpatient department failed to disclose any recurrence during the succeeding four years. Within the two months immediately preceding the final admission in November 1953, the patient began experiencing brief, transient syncopic attacks and increasing weakness. No tendency toward hemorrhage was present at this time.

On physical examination the patient was pale but did not seem acutely ill. Petechiae, purpura or bleeding from the mucous membranes was not present. The lymph nodes were not palpable. Gingival hyperplasia was not noted. There was a

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soft systolic murmur at the cardiac apex. The liver and spleen were not enlarged. The prostate was of normal size and consistency.

The hemoglobin was 6.25 gm. per 100 ml., the red cells 2.03 million per cu. mm. and the white cells totalled 3,100 per cu. mm., with segmented polymorphonuclears of 1 per cent, band forms 2 per cent, lymphocytes 19 per cent, metamyelocytes 1 per cent, myelocytes 3 per cent and "blast" forms 75 per cent. There were 73,000 platelets per cu. mm., and the reticulocytes numbered 2 per cent. The bone marrow aspirate showed a preponderance of peroxidase-positive blast forms, some with Auer bodies characteristic of acute granulocytic leukemia.

Urinalysis, serum bilirubin, acid and alkaline phosphatase, total protein, albumin and globulin and non-protein nitrogen were all normal.

Therapy was initiated with two units of red cell mass that raised the hemoglobin to 9 gm. The patient developed intermittent bleeding from the site of bone marrow aspiration. A moderate sized hematoma developed in the ear lobe which had been punctured by a blood lancet. Ecchymoses and hematomas developed in the subcutaneous tissues of the extremities. Five days after admission severe hematemesis and melena suddenly developed; the patient became comatose and died shortly thereafter.

Post-mortem examination showed leukemic infiltration into the spleen, liver and kidneys. During the period immediately preceding death massive hemorrhage into the gastrointestinal tract and the cerebral hemispheres developed. No gross or microscopic evidence for recurrent carcinoma of the bowel was noted. The prostate and pancreas were similarly free of neoplasm.

The coagulation time was performed by a standardized Lee-White method.[26] The bleeding time was determined by the Ivy technic.[27] Clot retraction and clot-lysis time were estimated by frequent periodic observation of the clot obtained in the coagulation time test, incubated at 37°C.

The fibrinolytic activity of the serum of the patient on a saline-washed clot obtained from a normal subject was determined by adding an amount of the patient's serum equal to the quantity of normal serum originally present. The tube was incubated at 37°C. and clot lysis noted by periodic inspection.

Fibrinogen was determined as outlined by Quick[28] in fresh plasma and in plasma incubated at 37°C. for twenty-four hours.

The prothrombin time, total prothrombin, prothrombin consumption time and thromboplastinogen activity time all were performed by methods originated by Quick.[28] The thrombin coagulation time was determined by estimating the coagulation time in seconds following addition of thrombin, 0.1 ml. to plasma 0.2 ml. (Table I.) The test for circulating heparinoids was carried out by adding protamine (0.5 per cent), 0.01 ml. to plasma, prior to determination of the thrombin coagulation time. Circulating anticoagulants were sought by mixing varying quantities of fresh whole blood of the patient with that from a normal control subject and determining the coagulation time. Following coagulation all of the tubes were observed for clot lysis.

TABLE I
TESTS OF HEMOSTATIC FUNCTION
(Coagulation time, 18 minutes; prothrombin time, 16.5 seconds; total prothrombin, 9 seconds)

	Minutes After Formation of Clot		
	15	30	45
Prothrombin consumption time (sec.):			
Tube I	12.5	12	11.5
Tube II	....	11	11
Thromboplastinogen activity time:			
Clotting time (sec.)	12.5	12	11.5

On the second hospital day the bleeding time was four and one-half minutes but hemorrhage from the site of puncture started again several hours later and continued for the remainder of the patient's course. The Lee-White coagulation time was eleven and one-half minutes. Clot retraction began in thirty minutes, but the entire clot was completely liquified within one hour. On the other hand addition of the patient's serum to the washed clot derived from normal blood had no such fibrinolytic effect.

The plasma fibrinogen concentration was determined to be 214 mg. per 100 cc. in freshly drawn blood. (Table II.) Although the fibrinogen concentration of a normal person determined at the same time was 337 mg. per 100 cc., a mixture of equal quantities of both plasma samples had a fibrinogen concentration of 272 mg. per 100 cc.

At the end of twenty-four hours of incubation at 37°C., the fibrinogen concentrations in these three tubes were as follows: patient's plasma, 182; control plasma, 214; and mixture of equal parts of both plasma samples, 219 mg. per 100 cc. In the tubes containing the plasma from the patient, the recalcified plasma clot observed at the thirty minute incubation period required for the performance of this determination was a brittle white flocculate and could be mobilized for sodium hydroxide digestion only by centrifuging. If recalcified plasma from the patient were allowed to stand longer, the clot would disappear entirely.

TABLE II
EFFECT OF INCUBATION UPON PLASMA FIBRINOGEN CONCENTRATION

Time (hr.)	Plasma Fibrinogen Concentration (mg. %)		
	Patient	Patient Control 1:1	Control
0	214	272	337
24	182	219	214

TABLE III
THROMBIN COAGULATION TIME

		Clotting Time	
		Patient (seconds)	Control (seconds)
Plasma	0.1 ml.	....	..
Thrombin 1/5	0.1 ml.	12	9
Plasma	0.1 ml.	....	..
Protamine (0.5%)	0.01 ml.	....	..
Thrombin 1/5	0.1 ml.	9.5	9

The prothrombin time was 16.5 seconds. Impaired prothrombin consumption (11 seconds) was very likely the result of thrombocytopenia, but impaired thromboplastinogen activity time (11.5 seconds) indicated deficiency in thromboplastic activity of the patient's blood. Thrombin coagulation times are listed in Table III. The prolonged clotting time of twelve seconds when thrombin was added to the patient's plasma indicated the presence of an antithrombic substance. Correction of this defect with protamine suggested that this substance was heparin-like in activity. This substance probably had no appreciable significance as a circulating anticoagulant because coagulation time of a normal person was not prolonged when the blood was mixed in varying proportion with that of the patient. (Table IV.) Although the patient's serum had no fibrinolytic effect on a normal washed clot, clot lysis took place following coagulation of mixtures of whole blood from that of the patient and that of a normal person. The Lee-White coagulation time repeated four days after hospitalization showed an increase to eighteen minutes.

TABLE IV
TEST FOR CIRCULATING ANTICOAGULANT

Patient's blood (ml.)	1.0	0.7	0.5	0.3	...
Control blood (ml.)	....	0.3	0.5	0.7	1.0
Coagulation time (min.)	18	7	7	6	5½
Fibrinolysis	+	+	+	+	...

COMMENT

Although thrombocytopenia played a contributing role, the data herein presented indicate that the chief cause of continued hemorrhage in this patient was destruction of the end product of coagulation, fibrin, by a proteolytic enzyme. The development of clinical events suggests that hemorrhage was a preterminal complication which led directly to death. There is no direct evidence to fix the exact time of onset of fibrinolysis in relation to hemorrhage or the basic disease. Symptoms suggesting leukemia were present for about two months and fibrinolysis was demonstrated at least two days before active hemorrhage had started and five days before death.

It is well known[29] that normal plasma contains an inactive precursor of fibrinolysin called "profibrinolysin." It is the potential function of this substance when activated to remove small blood clots following an inflammatory episode and to institute recanalization of clots in larger intravascular thrombi. The events leading to activation of profibrinolysin are not well understood. It has been postulated[30,31] that the tissues contain a profibrinolysin activator or "kinase" which initiates the changes leading to

clot dissolution in the event of injury, tissue breakdown, shock and other pathologic states.[32] That fibrinolysis may be inhibited by naturally occurring substances was suggested by Giraud et al.[21] and by our observations that a normal clot washed in saline was not dissolved by the serum of the patient. This observation probably represents a mechanism which in normal persons is protection against extensive fibrinolysis.

The possibility that the fibrinolysin in this case might also have fibrinogenolytic properties was suggested by diminished plasma fibrinogen values. (Table II.) Evidence of accelerated fibrinogenolysis, of necessity indirect, has been presented in previous *in vivo*[17] and *in vitro*[22,23] studies. It is our belief that a spurious diminution of plasma fibrinogen level might result from the action of fibrinolysin of the fibrin clot of recalcified plasma during the incubation period required by this examination. Also the drop of fibrinogen values from 214 to 182 mg. per 100 cc. following twenty-four hours' incubation of the patient's plasma was paralleled by a diminution of similar magnitude in the fibrinogen concentration of normal plasma incubated under similar circumstances. Thus our studies do not support the possibility that fibrinogenolysis occurred at least *in vitro*. More concrete evidence would have been derived from a study of the life span of transfused fibrinogen had this been done.

The prolonged prothrombin time in this case suggests that fibrinolysin might have destructive properties directed against prothrombin as well as other coagulation proteins. Stefanini and Gendel[33] have presented a case of carcinoma of the prostate which gave evidence that fibrinolysin was associated with marked shortening of the survival time of "purified" coagulation factors of various types. This point could not be investigated further in our patient due to his early demise. Study of the pathology[34] of the tissues removed at post-mortem examination failed to disclose any morphologic evidence of digestion of tissue proteins, suggesting that this enzyme did not possess a general proteolytic effect.

The paucity of published reports indicates that fibrinolysis is extremely rare in leukemia. In the authors' studies of forty cases of leukemia of various types[35] no other instance of fibrinolysis was demonstrated, nor was there any undue deficiency in the amount of plasma fibrinogen. In these cases of leukemia the level of the plasma fibrinogen was not correlated with the leukocyte count. Conditions accompanied by leukocytosis such as inflammation, on the other hand, are characteristically associated with an increase in the blood fibrinogen value. The studies of Georgeatsos and Quick[36] suggest that products of leukocytic breakdown, injected into experimental animals, are capable of stimulating the production of fibrinogen. Conversely the possibility that leukocytes, lymph nodes or bone marrow might actually manufacture or carry fibrinolytic enzymes[37] should be studied further.

The usual cause of hemorrhage in acute leukemia is thrombocytopenia associated with replacement of megakaryocytes in the bone marrow by leukemic cells. That thrombocytopenia also played a substantial role in bleeding in our case was evidenced by the appearance of spontaneous ecchymoses, gastrointestinal hemorrhage, low platelet count, absence of megakaryocytes and impaired prothrombin consumption.

Finally the presence of a circulating anticoagulant directed against thrombin was suggested by the increased thrombin time determination. The neutralization of this substance by protamine favored its heparinoid nature. Apparently this heparinoid substance was not present in sufficient quantity to cause a marked increase in coagulation time, nor did it prolong coagulation time of normal subjects.

SUMMARY

1. In an eighty-two year old man, acute leukemia was associated with fatal hemorrhagic phenomena. The chief factor in the mechanism of hemorrhage was destruction of the fibrin clot by a fibrinolytic enzyme.

2. The patient also showed thrombocytopenia due to absence of megakaryocytes, increase in prothrombin time, impairment of prothrombin consumption and increase of a heparinoid substance with antithrombic activity.

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Addendum: Since this paper was submitted for publication an account of fibrinolysis in acute granulocytic leukemia was published in the *Annals of Internal Medicine*, 42: 706, 1955 by Cooperberg and Neiman.

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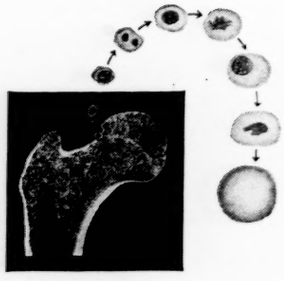

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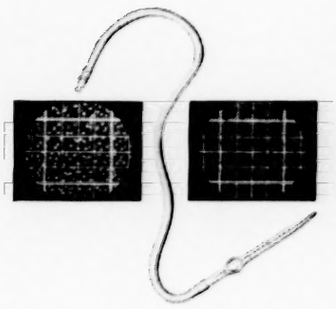
IN PREGNANCY—"...57 of the 58 patients (98.2 per cent) maintained or improved their hemoglobin [with Roncovite]."

—Holly, R. G.: Obstet. & Gynecol., *5*:562 (April) 1955.



IN CHRONIC LOW-GRADE INFECTIONS—"Cobalt appears to be a valuable drug in the treatment of anemias secondary to chronic diseases."

—Weinsaft, P. P., and Bernstein, L. H. T.: Amer. J. Med. Sc., Vol. 229, (Sept.) 1955.

"In all patients (chronic suppurative infection) a reticulocytosis was observed within 6 days. This was followed by increases in red-cell counts, in hemoglobin values, in blood volume and in total circulating hemoglobin."

—Robinson, J. C., et al.: New England J. M. *240*:749 (May) 1949.

Safe Medication

IN INFANCY—"There were no toxic effects in any case."

—Coles, B. L.: Archives of Disease in Childhood, *30*:150(April)1955.

"None of them [infants] showed harmful effects despite the large doses."

—Quilligan, J. J., Jr.: Texas St. J. Med. *50*:294 (May) 1954.

IN PREGNANCY—"No toxic manifestations associated with its use have been observed."

—Holly, R. G.: Obstet. & Gynecol. *5*:562 (April) 1955.

IN CHRONIC LOW-GRADE INFECTIONS—"With 60 mg. (cobalt chloride) a day by mouth after meals neither ourselves nor our patients experienced untoward symptoms."

—Robinson, J. C., et al.: New England J. M. *240*:749 (May) 1949.

"In our hands, cobalt appeared to be a useful and valuable drug, well tolerated and devoid of undue toxicity."

—Weinsaft, P. P., and Bernstein, L. H. T.: Amer. J. Med. Sc., Vol. 229, (Sept.) 1955.

AND . . . Thorough investigation has again verified the safety and lack of toxicity of Roncovite. Please refer to the four articles in the August 13, 1955 issue of the J.A.M.A. (Volume 158, No. 15) which fully document this convincing evidence.

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The original, clinically proved, pure *cobalt-iron product.*

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RONCOVITE TABLETS

Each enteric coated, red tablet contains:

Cobalt chloride	15 mg.
Ferrous sulfate exsiccated	0.2 Gm

Bottles of 100

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Each enteric coated red capsule-shaped tablet contains:

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Vitamin D	250 units

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Each 0.6 cc. (10 drops) provides:

Cobalt chloride (Cobalt 9.9 mg.)	40 mg.
Ferrous sulfate	75 mg.

Bottles of 15 cc. with calibrated dropper

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One tablet after each meal and at bedtime. Children 1 year or over, 0.6 cc. (10 drops); infants less than 1 year, 0.3 cc. (5 drops) once daily diluted with water, milk, fruit or vegetable juice.

Not enough hours in a day for him.
So he worries around the clock.
Soon his anxiety winds into a familiar pattern:
headaches, tension, nervousness, insomnia . . .
If he were your patient,
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Small dosage makes side effects rare . . . medication economical . . . dosage schedules simple. NEMBU-SERPIN is also recommended for treatment of mild essential hypertension.
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Tests for C-reactive protein depend on a single factor . . . the presence of inflammation.

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More Accurate than Sedimentation Rate Determinations[1]

"False positives" do not occur in tests for C-reactive protein because this abnormal protein appears only in patients with inflammatory conditions but is never present in normal serum.[2] Disappearance of CRP or changes in its concentration parallel more closely and more promptly variations in the patient's condition than usually evidenced by fluctuations in sedimentation rate.

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More Easily Interpreted than Sedimentation Rate Determinations[3]

There is no "normal," therefore no equivocal zone of values in the interpretation of tests for C-reactive protein.[4] CRP tests are not invalidated in patients with congestion of the liver, in heart failure, anemia, cyesis, nephrotic syndrome, or changes in fibrinogen, serum albumen or globulin, when sedimentation rates may be misleading.

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More Convenient to Perform than Sedimentation Rate Determinations[5]

The test for C-reactive protein may be performed at any time after obtaining a sample of the patient's serum. Since a large volume of serum is not necessary, blood may be drawn from a fingertip rather than from a vein. The simple technique employed in CRP determinations facilitates *routine* use in office and hospital.

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C-reactive Protein Antiserum (Schieffelin)

For complete descriptive brochure on techniques and materials required, send request to:

Schieffelin & Co. New York 3, N. Y. • Pharmaceutical and Research Laboratories since 1794

1. Shackman, N. H.: Heffer, E. T., and Kroop, I. G.: Am. Heart J. 48:599 (Oct.) 1954. • 2. Stollerman, G. H., and others: Am. J. Med. 15:645 (Nov.) 1953. • 3. McEwen, C.: M. Clin. North America 39:353 (March) 1955. • 4. Wood, H. F., and McCarty, M.: Am. J. Med. 17:768 (Dec.) 1954. • 5. McEwen, C., and Ziff, M.: M. Clin. North America (May) 1955, to be published.

Latest data on **effectiveness** of Furadantin®

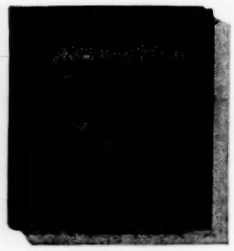
brand of nitrofurantoin, Eaton

in urinary tract infections

Investigators:

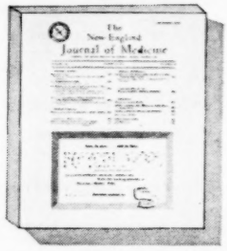
Latest data on effectiveness of Furadantin

Flippin, H. F., and Eisenberg, G. M.: Antimicrobial Therapy in Medical Practice, Philadelphia, F. A. Davis Co., 1955, p. 40.



Clinical studies have demonstrated rapid clinical response in cases of cystitis and pyelonephritis, including infections caused by refractory organisms.

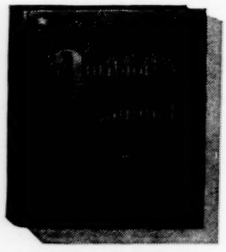
Trafton, H. M., et al.: New England J. Med. **252:** 383, 1955.



13 acute cases . . . 6 appeared cured . . . 6 markedly improved with no relapses.

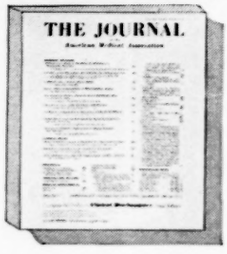
36 chronic infections: 30 showed symptomatic improvement, frequently within 24 hours.

Beutner, E. H., et al.: Antibiotics Annual, 1954-1955, New York, Medical Encyclopedia, Inc., 1955, p. 988.



30 chronic urinary tract infections: Of 47 strains of bacteria isolated from these patients, 29 strains (62%) were eradicated by Furadantin.

Hasen, H. B., and Moore, T. D.: J.A.M.A. **155:** 1470, 1954.



Of patients with acute urinary tract infections, 95.7% were benefited. Patients with chronic infections and those with organic or obstructive lesions were benefited in 82% of cases.

Dosage — average adult: four 100 mg. tablets daily, 1 tablet with each meal and 1 tablet on retiring, with food or milk.

Furadantin tablets, 50 and 100 mg. in bottles of 25 and 100. Furadantin Oral Suspension (5 mg. per cc.), bottle of 4 fl.oz. (118 cc.).

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—the first choice of cough suppressants, highly effective, yet non-addictive.

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REFERENCES:

1. Blanchard, K. and Ford, R. A., Effective Antitussive Agent in the Treatment of Cough in Childhood, Journal-Lancet, 74:443, Nov., 1954.* **2.** Cass, L. J. and Frederik, W., Comparative Clinical Effectiveness of Cough Medication, Amer. Pract. and Dig. of Treat., Vol. 2, p. 844, October, 1951.*

**Reprints available upon request.*

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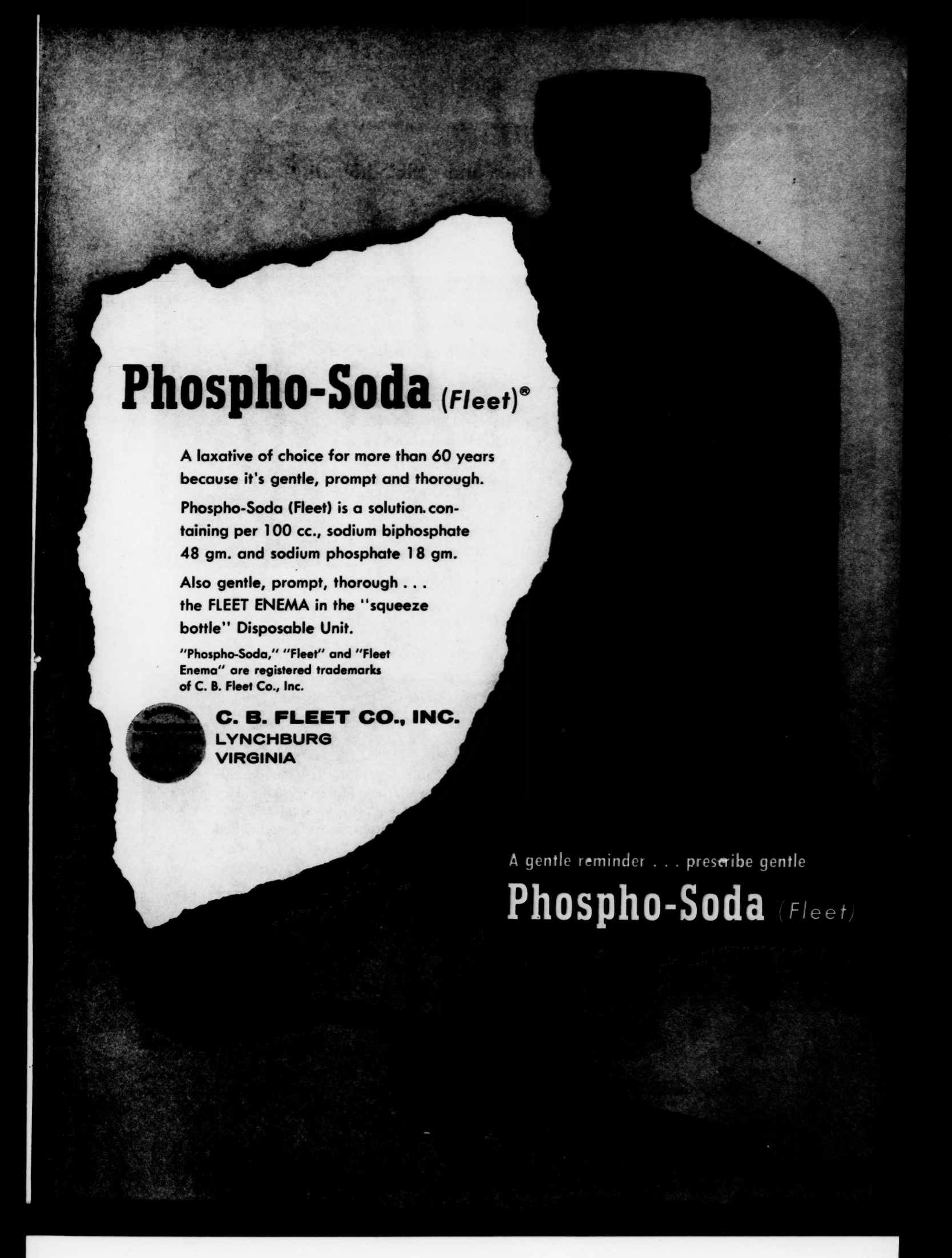
1. Overall, J. C.: Southern M. J. 47:789, 1954. 2. Editorial: New England J. M. 246:111, 1952.
3. Grayzel, H. G., Heimer, C. B., and Grayzel R. W.: New York St. J. M. 53:2233, 1953.
4. Heimer, C. B., Grayzel, H. G., and Kramer, B.: Archives of Pediatrics 68:382, 1951.
5. Behrman, H. T., Combes, F. C., Bobroff, A., and Leviticus, R.: Ind. Med. & Surg. 18:512, 1949.
6. Turell, R.: New York St. J. M. 50:2282, 1950.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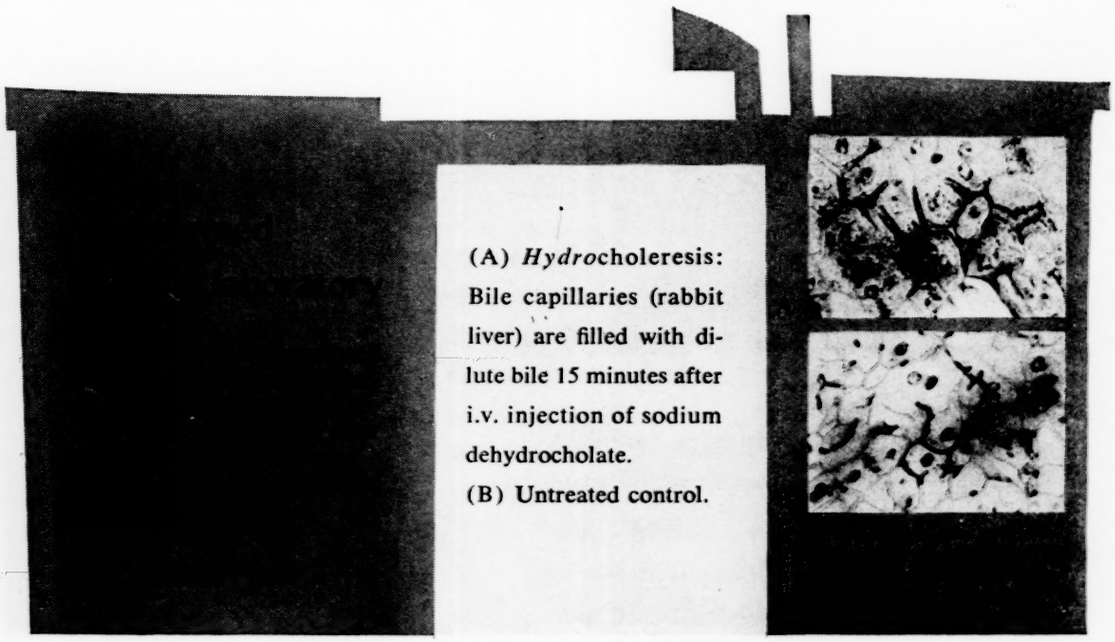

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(1) Clara, M.: Med. Monatsschr. *7*:356, 1953. (2) Brauer, R. W., and Pessotti, R. L.: Science *115*:142, 1952. (3) Schwimmer, D.; Boyd, L. J., and Rubin, S. H.: Bull. New York M. Coll. *16*:102, 1953.

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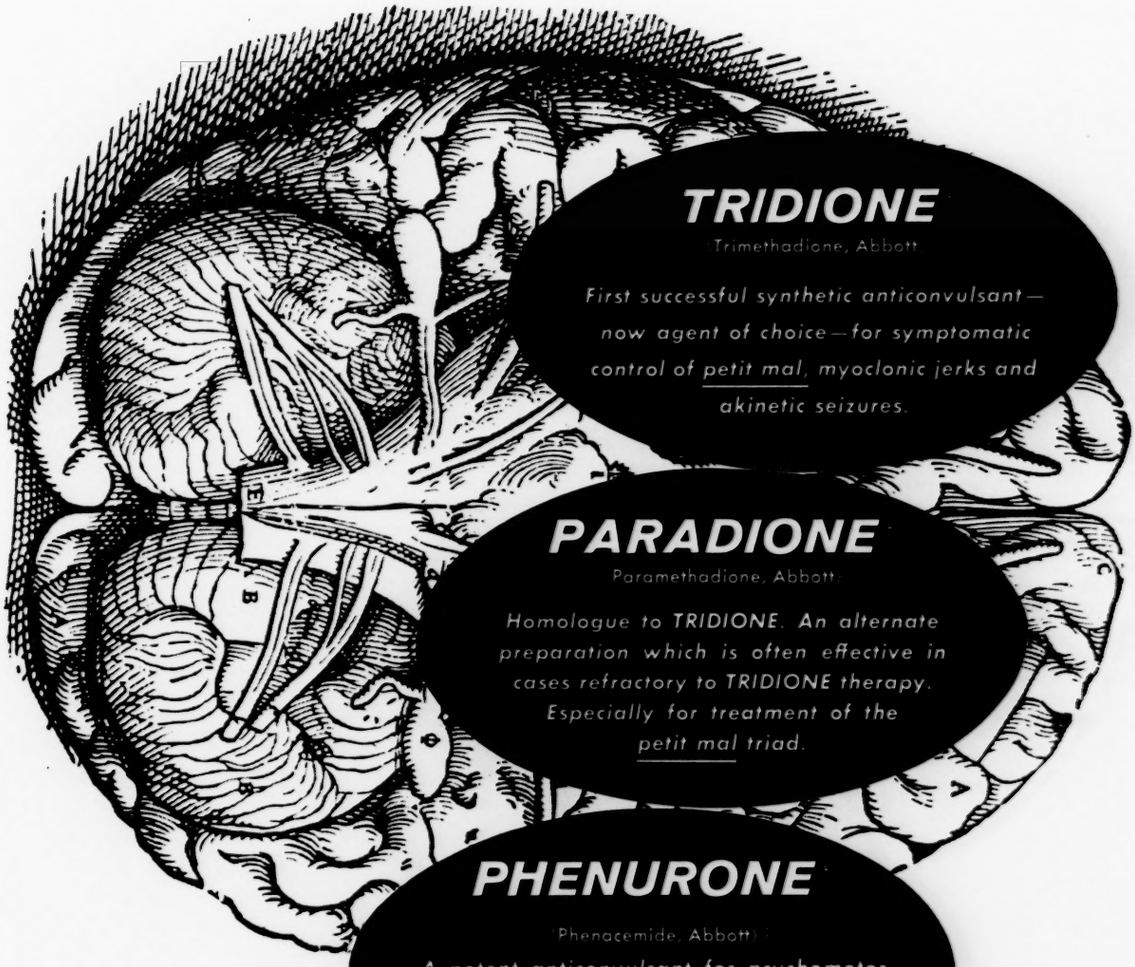

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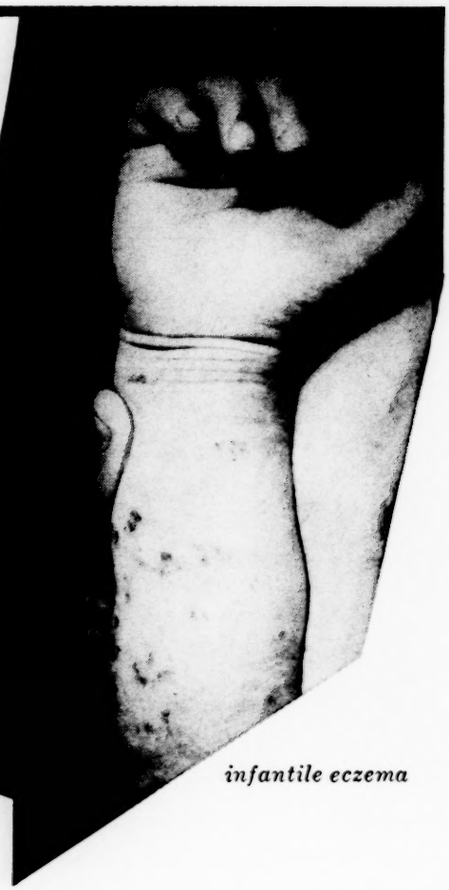

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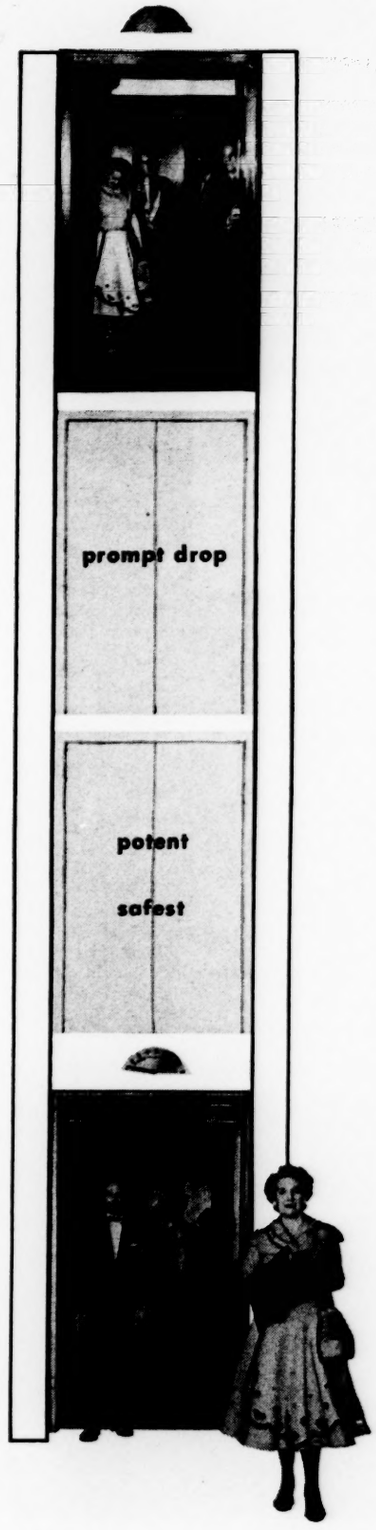

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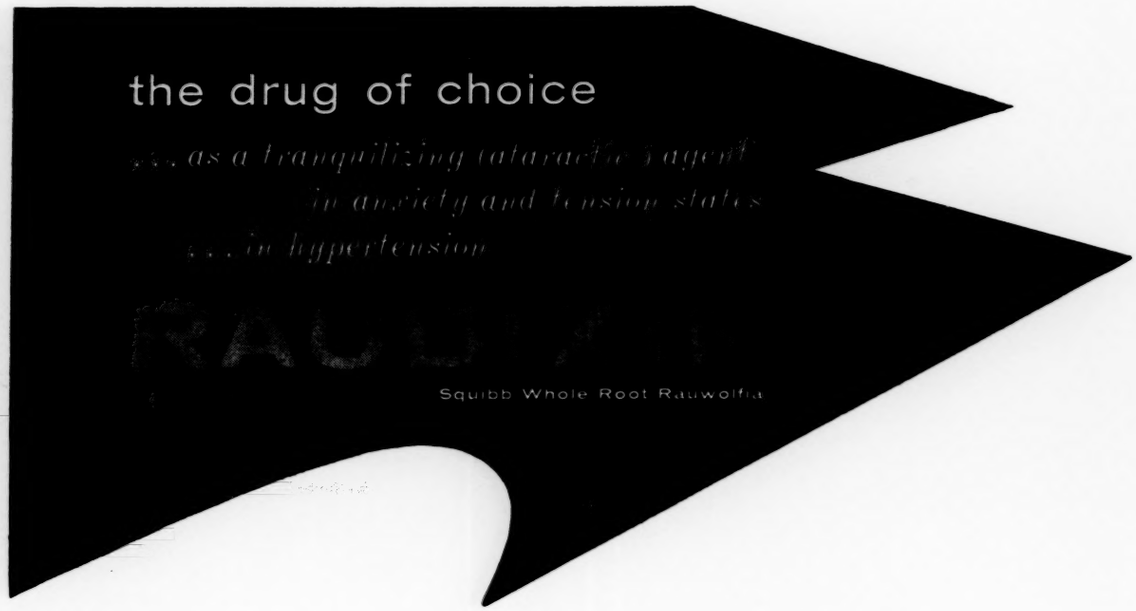

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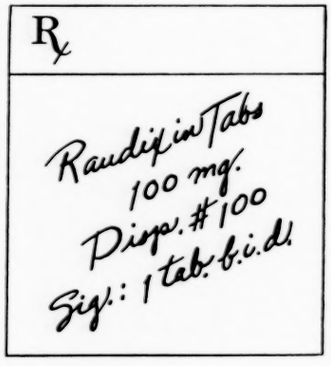

*Ataractic, from ataraxia: calmness untroubled by mental or emotional excitation. (Use of term suggested by Dr. Howard Fabing at a recent meeting of the American Psychiatric Association.)

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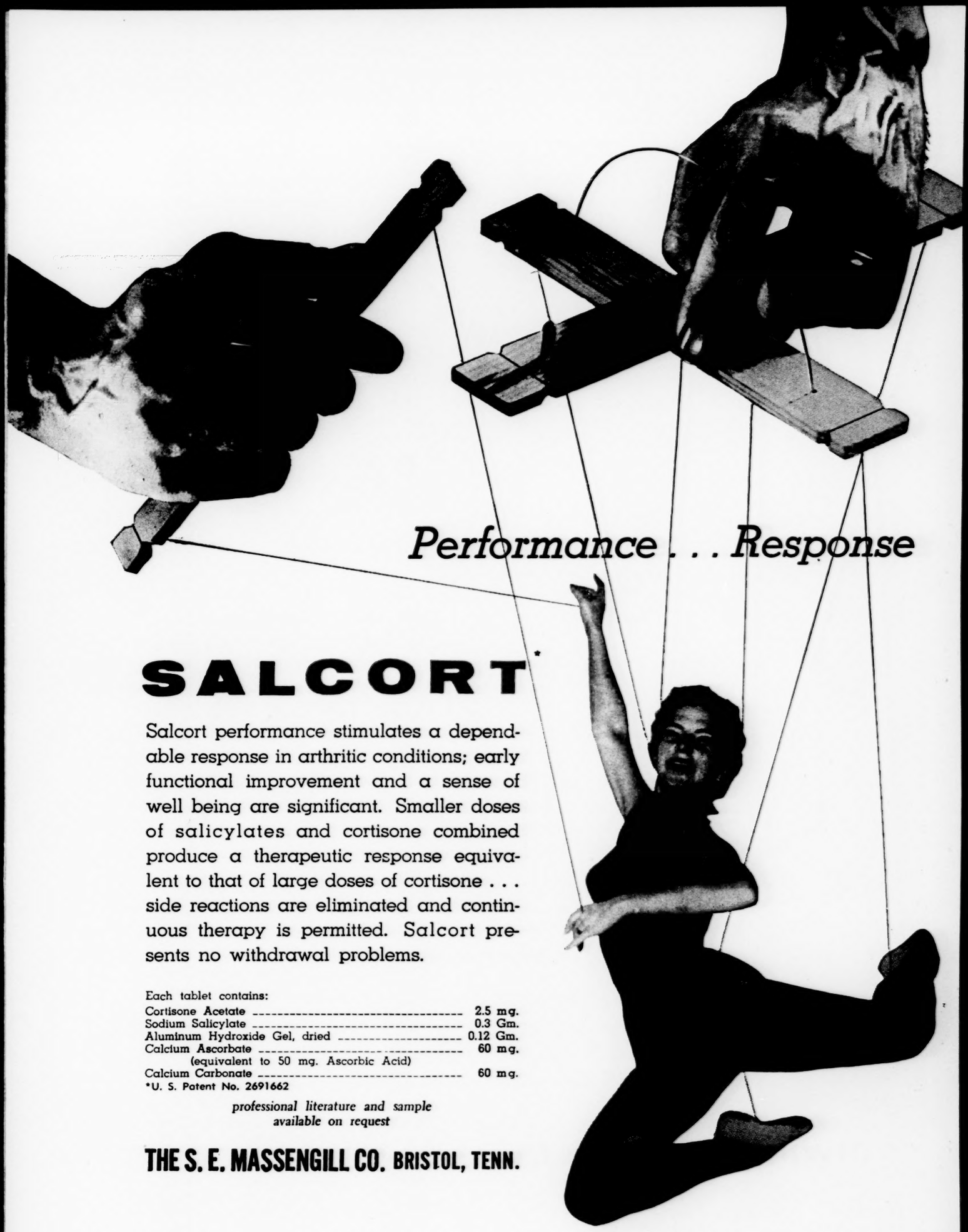

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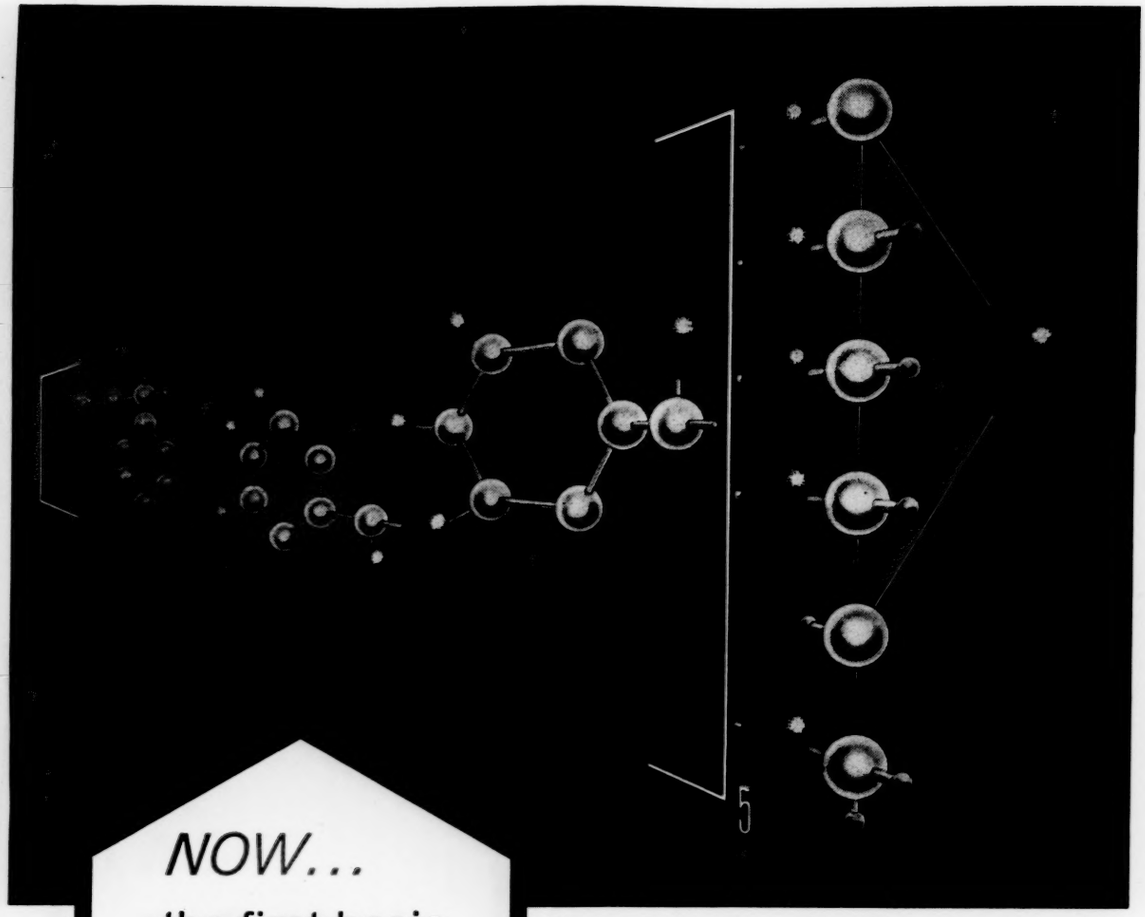

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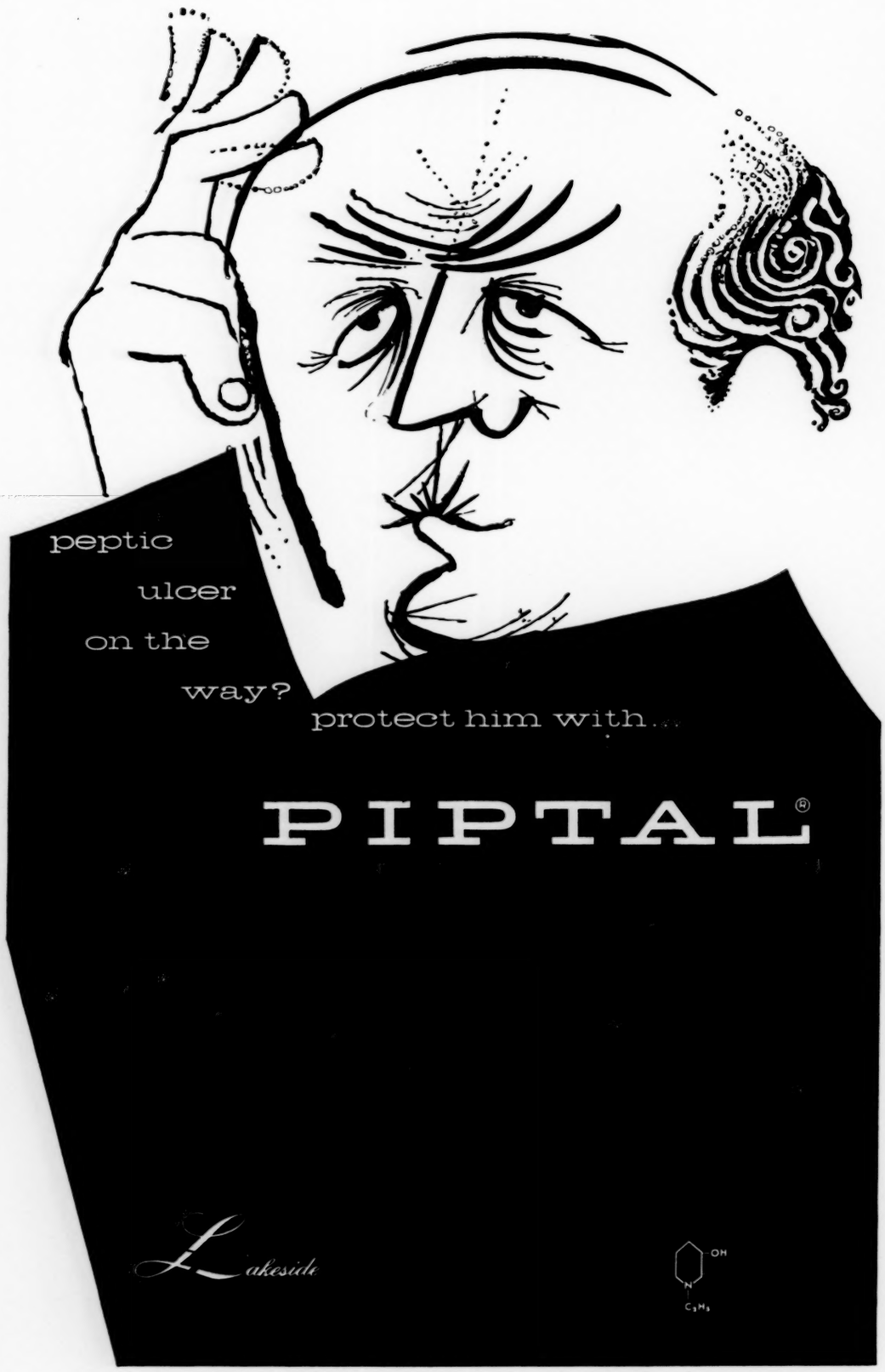
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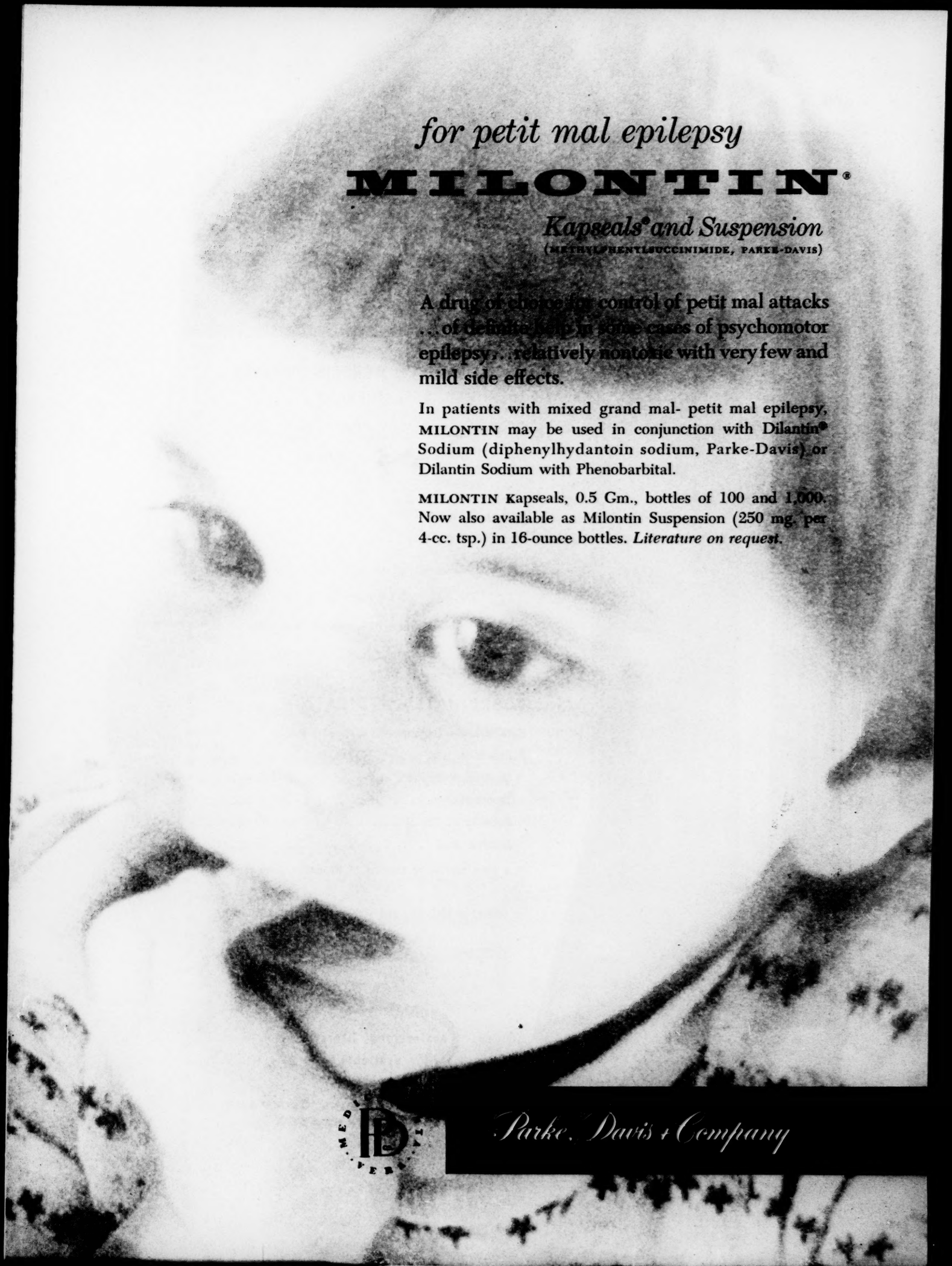

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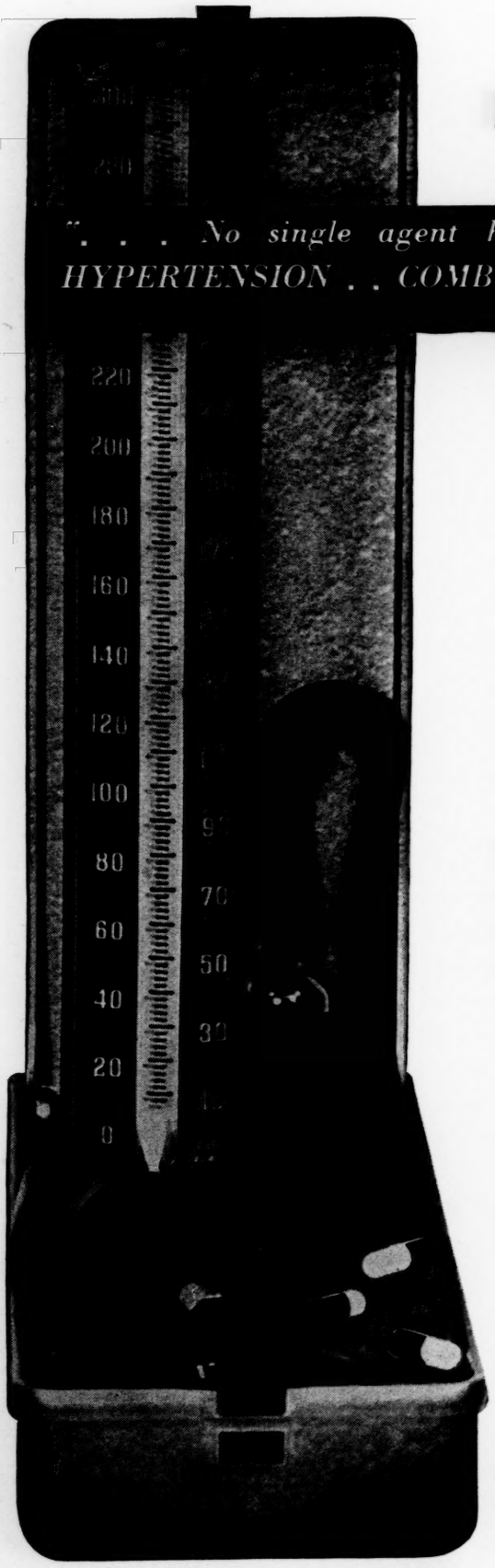

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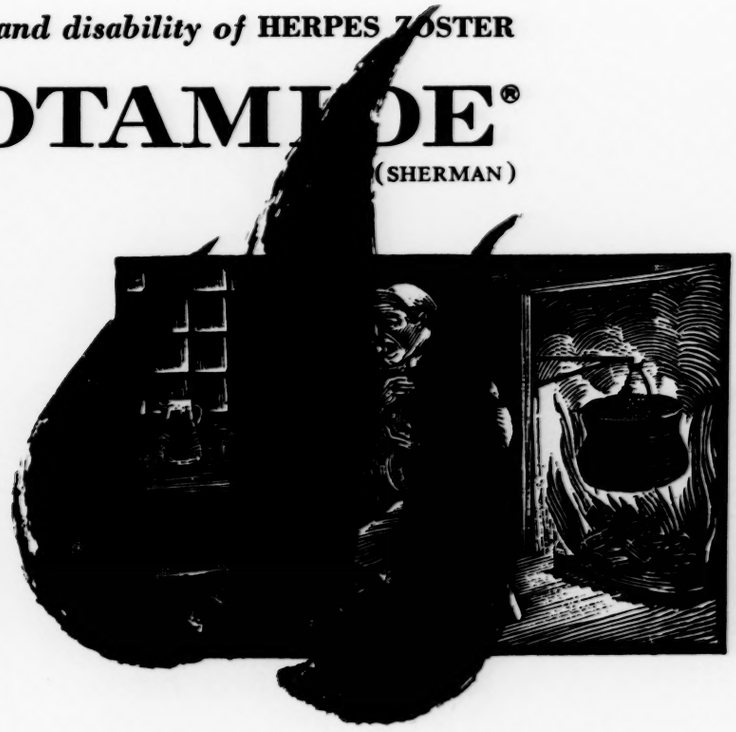

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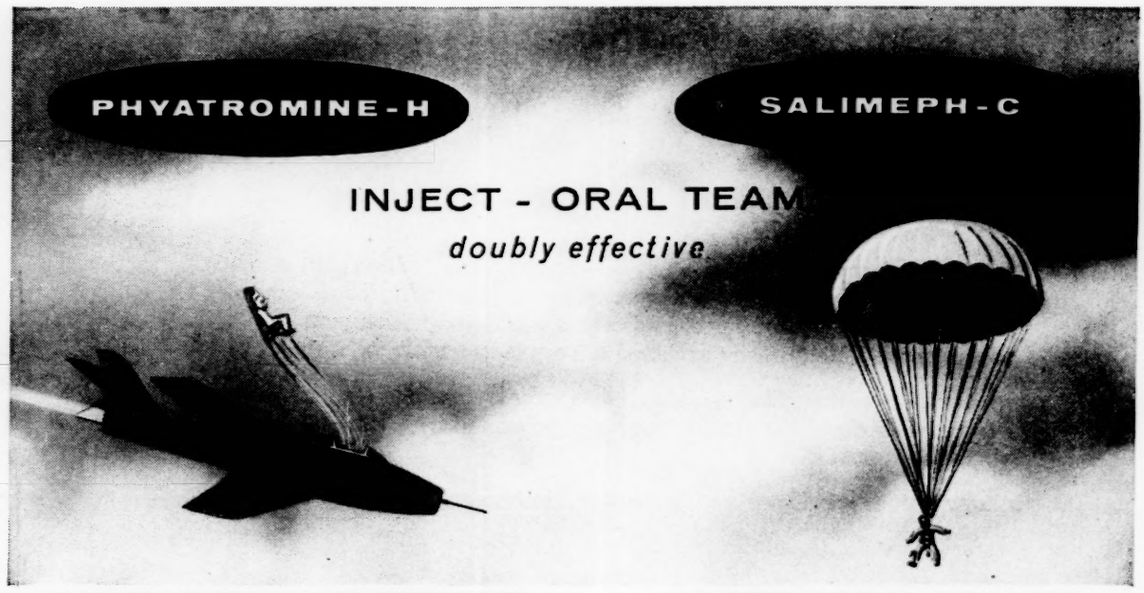

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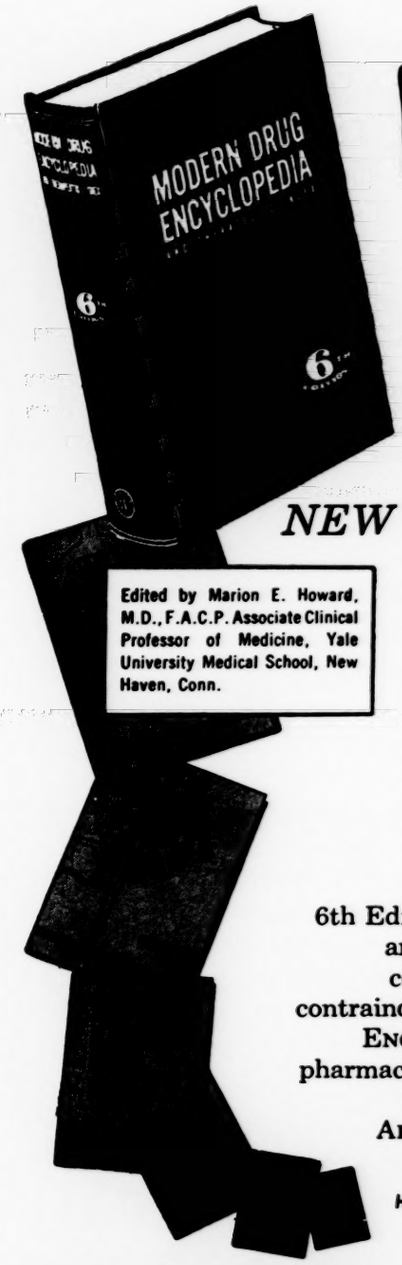

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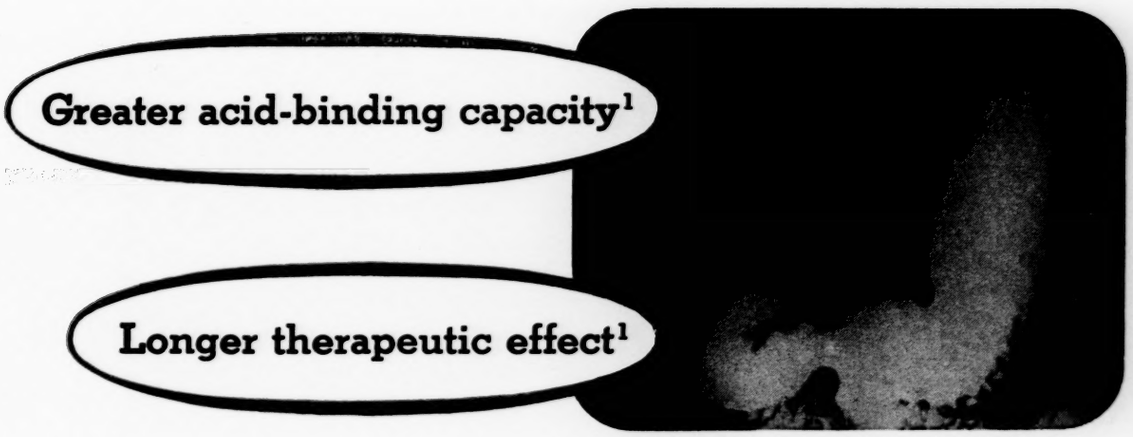

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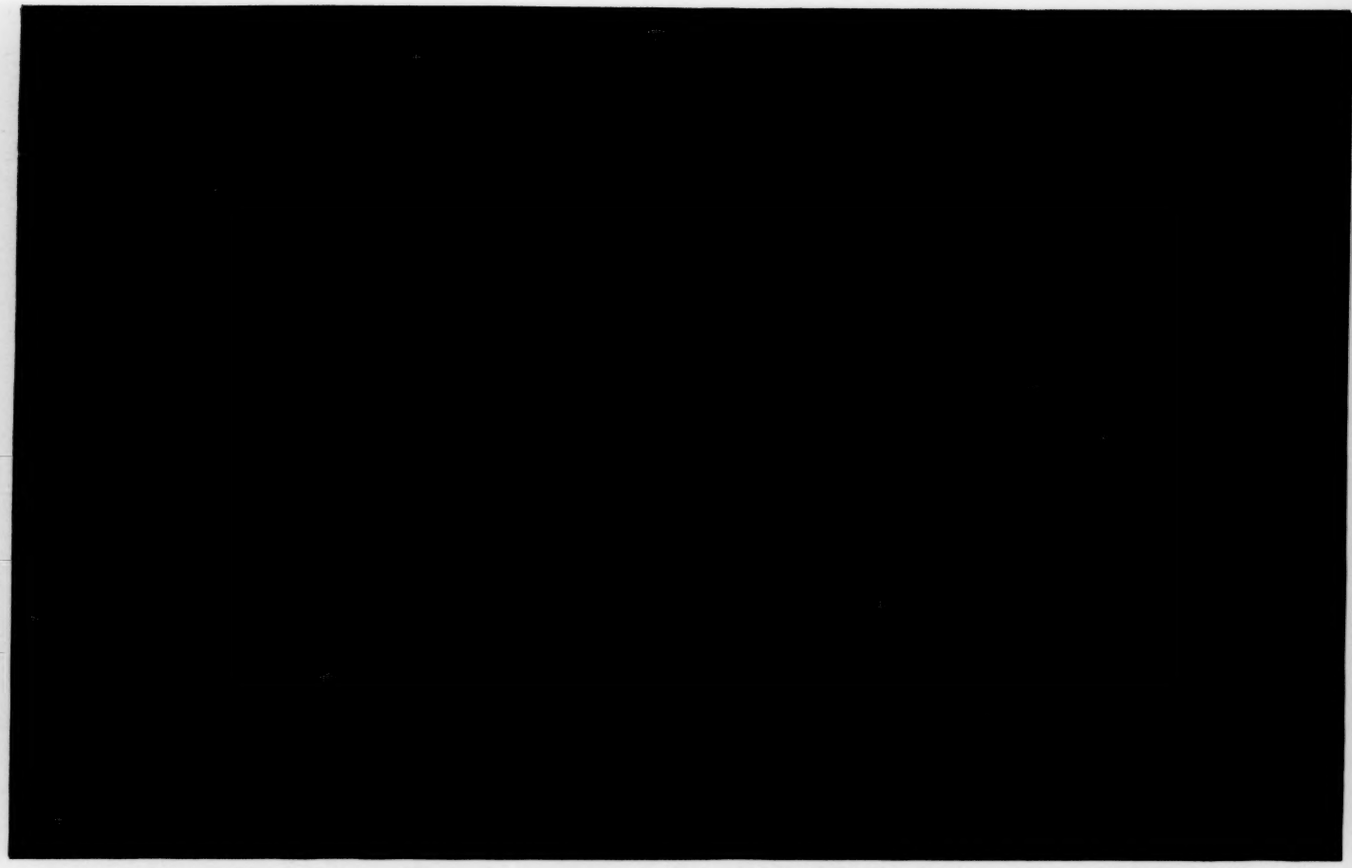
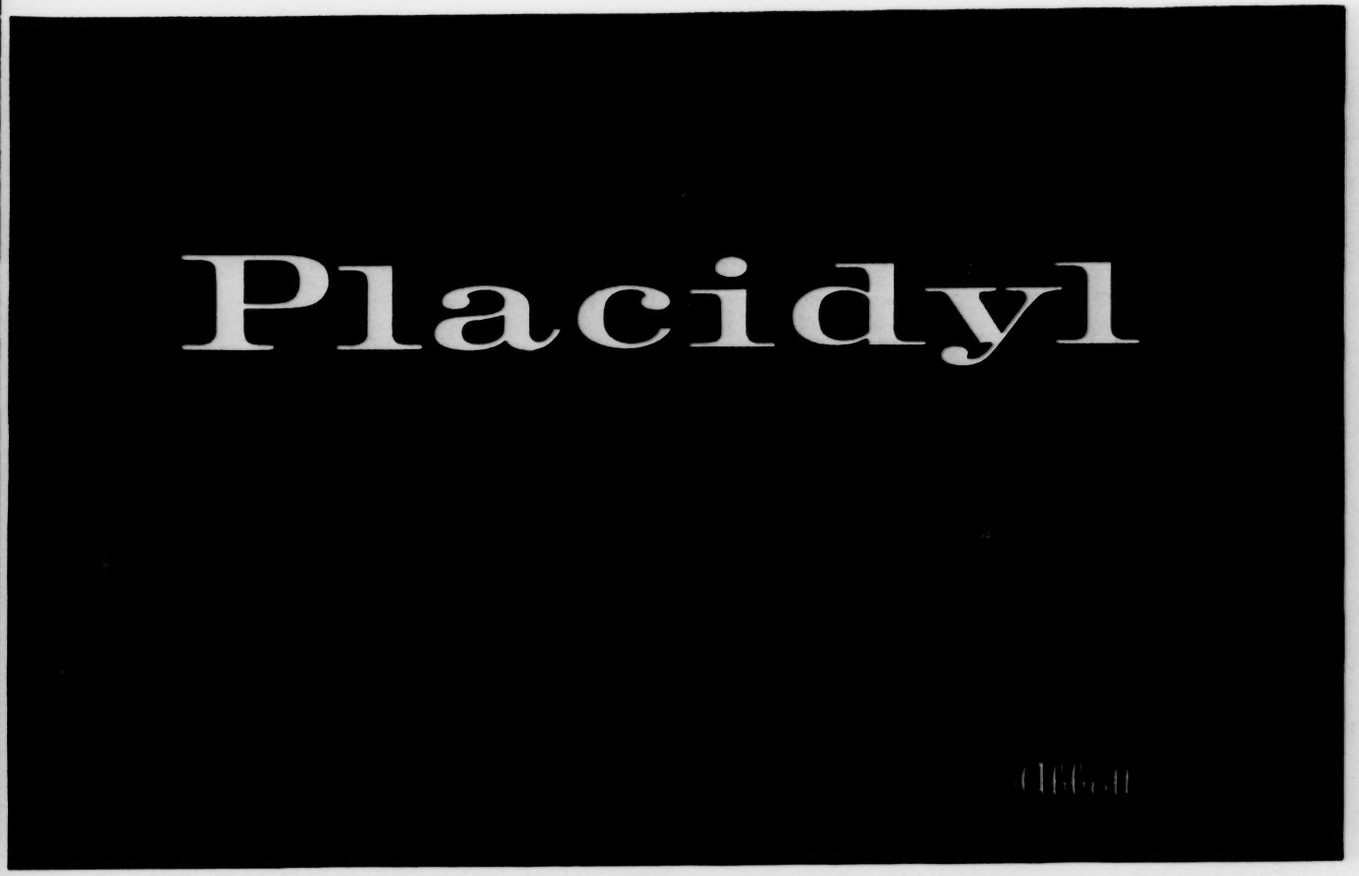
2. Morrison, Samuel: Magnesium Aluminum Hydroxide Gel in the Antacid Therapy of Peptic Ulcer, Am. J. Gastroenterology 22:309 (1954).

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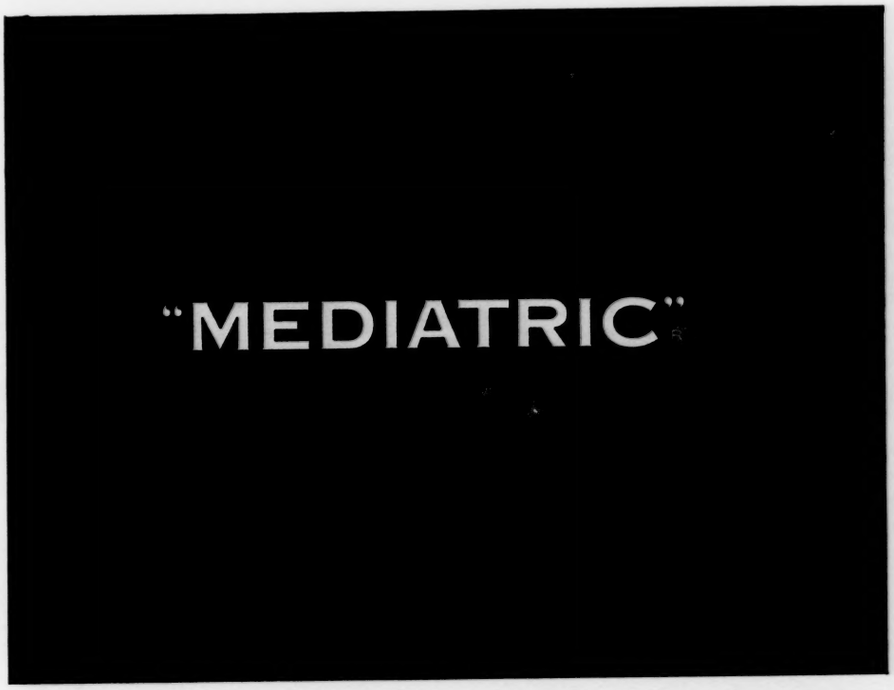
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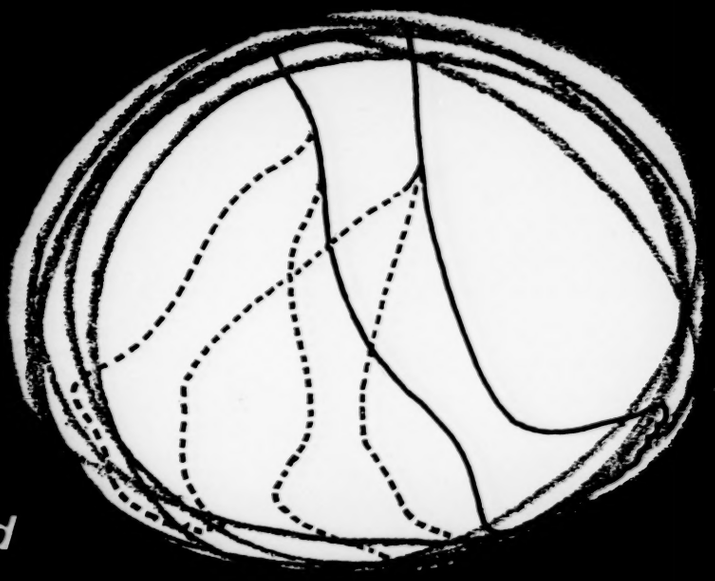

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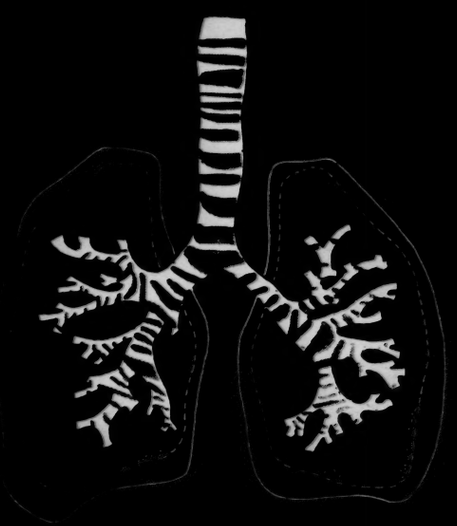

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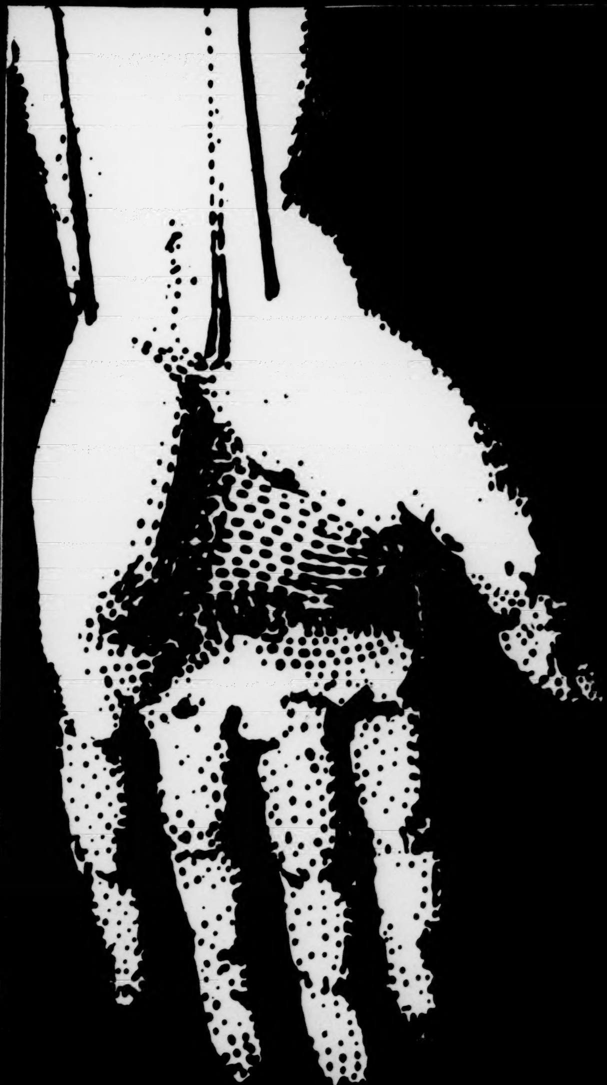

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